COLD-INDUCED HYPERTENSION IN RATS

By

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This dissertation is dedicated to my family, and
especially to my father, John Papanek Jr.
whom I miss dearly.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

ABSTRACT vi

CHAPTER ONE BACKGROUND 1

Cold Research 1
Role of the Sympathetic Nervous System in Adaptation to Cold 5
Hypertension 8
Cold Hypertension as a Model of Essential Hypertension 14

CHAPTER TWO COLD-INDUCED HYPERTENSION IN RATS 16

Introduction 16
Methods 18
Results 20
Discussion 31

CHAPTER THREE THE EFFECT OF A LOW SODIUM DIET ON COLD-INDUCED HYPERTENSION 40

Introduction 40
Methods 41
Results 43
Discussion 56

CHAPTER FOUR THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN COLD-INDUCED HYPERTENSION 64

Introduction 64
Methods 67
Results 73
Discussion 89

CHAPTER FIVE THE ROLE OF VASOACTIVE HORMONES IN COLD-INDUCED HYPERTENSION 102

Introduction 102
Methods 104
Results 107
Discussion 117
Summary 128

CHAPTER SIX SUMMARY 129

APPENDIX A EXTRACTION OF PLASMA CATECHOLAMINES 140

APPENDIX B ARGININE VASOPRESSIN RADIOIMMUNOASSAY 142

APPENDIX C PLASMA RENIN ACTIVITY ASSAY 144

APPENDIX D CORTICOSTERONE RADIOIMMUNOASSAY 147

REFERENCES 149

BIOGRAPHICAL SKETCH 163

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Chronic exposure of male rats to low environmental temperature (6°C) results in the development of hypertension which is characterized by an increased mean arterial pressure (124 ± 3 mm Hg) and cardiac hypertrophy. The onset occurs within 2 weeks, is repeatable, and results in a systolic blood pressure of 150 ± 3 mm Hg after 42 days of exposure to cold whether measured in cannulated animals or indirectly by the tail-cuff technique. The concentrations of certain vasoactive agents in the plasma of resting animals whose femoral artery was chronically cannulated strongly suggest a large neurogenic component in this model. Thus, the concentration of norepinephrine in plasma was significantly increased throughout the duration of exposure, while the concentrations of epinephrine and corticosterone in plasma increased initially, but returned to basal levels within 2 weeks. Similarly, plasma renin activity increased initially, but was returned to the level of controls at 20 days. The concentrations of aldosterone, sodium, potassium, and arginine vasopressin in plasma and osmolality of serum were unchanged. The sensitivity of the baroreceptor reflex was decreased, this suggests an alteration in the central processing of sensory (blood pressure) information. Food intake, urine output, and hematocrit were significantly increased, while water intake remained unaffected, suggesting a self-imposed volume contraction. The effect of cold does not appear to be unique to the rat as there is evidence to suggest that low environmental temperature adversely affects blood pressure in humans as well.

Although the exact mechanism for cold-induced hypertension remains undetermined, it can be concluded that chronic exposure to cold represents a unique method for the induction of hypertension which does not involve either surgical intervention or the administration of pharmacological doses of a drug. The results support the hypothesis of a neurogenically-induced, renal-mediated form of hypertension which may involve the central and peripheral renin-angiotensin II system. Additional work will be required to determine the exact mechanisms involved in cold-induced hypertension.

CHAPTER ONE
BACKGROUND

Exposure of either rats or humans to low environmental temperatures results in a variety of physiological responses aimed at maintaining body temperature either at or near normal. The investigation of the physiological responses induced by changes in environmental temperature is not a new area to physiologists. Indeed, physiologists have been interested for many years in both the acute and chronic changes which affect survival. The information relating to the regulation of body temperature is so vast that textbooks must devote entire chapters to the subject to ensure its adequate review.

Cold Research

Among the first studies utilizing cold were those performed in the early 1870s by Claude Bernard (13). In these classical studies, Bernard suggested the dependence of heat production on two primary sources, muscular activity, and chemical processes that are not involved in muscular activity. Both are ideas which continue to provoke considerable research. According to Bard (6), another very early idea which was later "rediscovered" is that of Ott who, in 1887, suggested a hypothalamic center for temperature regulation. This idea was essentially ignored for over 50 years. In 1888, Hoesslin (94) described the adaptation of small animals to a reduced temperature as a slow process which involved the formation of a protective coat of fur and fat for insulation. This mechanism for induction of adaptation is one displayed by many mammalian species. Isenschmid and Krehl (98) in 1912 concluded that the diencephalon was the region which was important in temperature regulation. Sherrington (177), demonstrated that the essential temperature regulatory center was located prespinal and showed that decerebrate animals effectively became poikilothermic. These very early studies, when physiology was a young science, were involved in describing the behavioral responses to cold exposure and were primarily associated with the very basic ideas of control by the central nervous system.

It appears that the pioneering investigations of Cannon and his associates (22) in the mid 1920s were the first series of studies which undertook a systematic investigation of the physiological mechanisms involved in maintaining temperature by chemical means. The major emphasis in these studies was to determine the function of the adrenal medulla and to understand how its secretion was controlled. Cannon et al. (25) demonstrated that the output from the adrenal gland was not fixed or tonic but rather variable. In addition, it was controlled by the sympathetic nervous system (SNS). Cannon et al. (24) were the first to use sympathectomized cats with respect to responses to cold. Behaviorally, these cats were poikilothermic; much like Sherrington's (177) decerebrate animals. They "lived" on the room heaters, leaving mainly for food. This suggests an inability either to generate heat internally or to prevent loss of body heat. In addition, these cats demonstrated a greater decrease in core temperature when challenged by cold. These studies suggested a major role for the sympathetic nervous system (SNS) and adrenal medulla in the mechanisms for defence against low temperature. In time, Cannon et al. (25) described the role of an adrenal medullary secretion which increased the rate of oxidation in tissues and provided substrates for the oxidative processes. Hartman et al. (82), as well as Cannon, demonstrated an increase in the release of a substance (later shown to be epinephrine) from the adrenal medulla when the animals were exposed to cold. These, as well as other studies, led Cannon (23) to conclude that the adrenal substance was the primary mediator in the chemical control of body temperature. It was during this period of time that Cannon also demonstrated that this adrenal substance played a significant role in the generalized response to stress.

During the 1930s a major emphasis was directed toward the localization of centers within the central nervous system which influenced both heat production and heat loss, and in establishing critical body temperatures for survival. The general emphasis in physiology at this time was on the concepts of neurotransmission, chemical mediators and neurocontrol centers. Ranson (161) formulated the idea of two distinct hypothalamic centers, one which responded to heat and one which responded to cold. Ranson noted that the neurons which made up the primary area responsible for the protection from cooling were distributed though a large part of the hypothalamus and may be in part, identical with those regions involved in controlling vasoconstriction, piloerection and some other sympathetic functions (161). Thus, the center which responds to low temperature is not a discrete nucleus, but rather a diffuse collection or series of neurons localized within the hypothalamic region. Further, it appears that there is a significant amount of overlap with respect to anatomical location and the control of physiological responses. It is well known that this same region serves a variety of other key autonomic functions including feeding, drinking, origin of circadian rhythms, and control of blood pressure.

From 1940 to the early 1970s a tremendous amount of research was conducted in environmental physiology and the physiological responses to cold. Perhaps some initiative was contributed by the problems generated by World War II and the military. A great deal of impetus was also generated by the rapid advances made in the areas of electronics as well as in laboratory techniques, such as the development of spectroscopy, chromatography, recording equipment, computers, and radioimmunoassays. Many studies investigated the influence which changing environmental temperature and altitude had on various factors including: the circulatory, pulmonary and endocrine systems, as well as internal temperature, metabolic rate, and survival. The classical study by Herrington (89) demonstrated an increased metabolic rate in small laboratory rats, mice, and guinea pigs in response to a decreased environmental temperature. The author (89) measured thermal conductances, skin and internal temperatures, and heat production over a wide range of ambient temperatures. In accordance with Herrington's equation for calculating the increase in heat production with decreasing environmental temperatures, an increase of 157% above basal would occur at 6°C, in the case of the rat (89). The changes in metabolic rate demonstrated by Herrington in 1940 are striking and have been duplicated repeatedly as techniques for measuring metabolic rate in laboratory animals and humans have improved. The elaborate balance involved in maintaining core temperature has been described by DuBois (44). Heat producing factors (basal metabolic rate, specific dynamic activity of food, moderate activity and exercise, or shivering) must be equally offset by heat loss mechanisms (basal heat loss, increased peripheral cooling, and sweating) if normal temperature is to be maintained. When any factor is changed, the balance is temporarily altered until the opposing mechanisms which are capable of restoring equilibrium are engaged.

Utilizing the basic scheme as described by DuBois (44), it can be seen that the ability to maintain body temperature in response to cold is thus dependent on both a decrease in heat loss and an increase in heat production. Increased heat production is accomplished initially by shivering and an elevation of basal metabolic rate (BMR). This increase in metabolic rate can be dramatic as indicated above. As exposure to cold continues, the emphasis is shifted away from mechanical heat producing mechanisms, i.e. shivering, and towards biochemical mechanisms. Although shivering ceases, body temperature is maintained by "nonshivering" thermogenesis (NSTG), i.e. a biochemical process of heat production. It is this NSTG which has generated many questions. What is the stimulus for NSTG to begin functioning? Which animals possess this mechanism? Where is the site of NSTG? How is NSTG regulated? What hormonal systems are involved in this response?

A great deal of research has been directed toward each individual hormonal system as it is related to acute cold exposure, NSTG, and adaptation. A thorough review of any of these systems is beyond the scope of this dissertation, and further, each has been the subject of published reviews. The phasic responses of the major hormones, their interactions to provide additional substrate for heat production, and the subsequent increase in systemic responsiveness of thermogenic mechanisms have been reviewed elsewhere (57,59,92,141). However, the role of the sympathetic nervous system merits a brief review.

Role of the Sympathetic Nervous System in Adaptation to Cold

In 1942, VonEuler (194) isolated norepinephrine (NE) and quickly developed a bioassay. In 1946, VonEuler (194) predicted that NE was the neurotransmitter substance of the SNS. It was nearly 10 years later that this idea was documented when fluorescence histochemical techniques became available (194). In 1955, VonEuler and Floding (195) developed a fluorimetric technique for measuring both NE and E in urine. The ability to measure NE and E concentrations resulted in a major stimulus for research in the role of the sympathetic nervous system in thermoregulation.

When animals are exposed to cold, the SNS is markedly stimulated. That this occurs is evident by a significantly increased excretion of catecholamines (NE and E) in a variety of species including rat (133,121,122) and man (6,183). Although a great deal of variability exists in the actual amount of catecholamines excreted, the cold-induced increases were large in all cases. The variations in the results can be attributed to differences in assay techniques, temperature, and the age of the animal. In addition, the fluorimetric technique is not sufficiently sensitive to detect the low concentration (pg/ml) of catecholamines in the plasma at rest. The variation attributed to assay techniques is no longer a problem due to the development of high performance liquid chromatography (HPLC) and radioenzymatic (RE) techniques for measurement of plasma, urinary, and tissue catecholamines. These newer techniques have both greater sensitivity and decreased variability. The major difference between these two techniques (HPLC and RE) is not attributed to the precision, but rather one of methodologies. The size of the sample required is quite different (HPLC at least 0.5 ml vs 0.05 ml for radioenzymatic) as is the cost per sample (HPLC significantly less). The size of the sample of plasma necessary for reliable analysis becomes an important consideration when the protocol involves either a small animal model or serial sampling. This is often the limiting factor in the design of an experiment in which the concentration of catecholamines in the plasma is to be determined. The concentrations of catecholamines in plasma are a more accurate representation of temporal changes within the organism, whereas the concentrations of catecholamines in urine represent both sympathetic and central nervous system activity over an extended period. Unless sympathetic changes can be sustained for long periods of time, the concentration of catecholamines in urine are difficult to interpret. The concentration of catecholamines in the urine is affected not only by the concentration of catecholamines in the blood, but also by the factors which influence their metabolism and renal clearance. The development of high pressure liquid chromatographic technique for measurement of catecholamines has contributed to a renewed interest in the area of sympathetic function during exposure to cold.

As mentioned above, adaptation to cold is characterized by a markedly increased thermogenesis. The increased nonshivering thermogenesis (NSTG) is attributed to both changes in heat producing mechanisms (88) and a supersensitivity to the metabolic effects of NE (41,96). Hsieh and Carlson (97) were among the first to demonstrate an enhanced calorigenic response to administered (i.m.) catecholamines. The metabolic response to norepinephrine was much greater than that to E given i.m. This led these authors to conclude that NE was the primary mediator of the enhanced calorigenic response. Depocas (41) demonstrated an enhancement of the calorigenic response to NE infused intravenously in cold-acclimated rats. These results also lead Depocas (41) to conclude that NE, and thus the sympathetic nervous system, is primarily responsible for the markedly increased rate of oxygen uptake ($\dot{V}O_2$) seen with exposure to cold. However, when infused intravenously, E results in an equally large calorigenic response (91). The work of several investigators suggested that NE was the primary mediator of NSTG while E served multiple functions, including supplying substrates for metabolism and acting as a backup to NE, if needed. For instance, when adrenalectomized rats (glucocorticoids replaced) are exposed to cold, no increase in E is detected (170). Norepinephrine responses are unaffected by adrenalectomy (100). Various attempts have been made to eliminate the NE response to cold by immunosympathectomy, ganglionic blockade, adrenergic blockade, and NE depletion. Although each of these techniques has limitations, it can be concluded that when NE responses are limited, E release is enhanced when possible. Based on such studies, it would appear that NE, and thus the SNS, is primarily responsible for the markedly increased $\dot{V}O_2$ seen during exposure to cold as concluded by Hsieh and Carlson (97), and Depocas (41). Further, E serves the multiple functions of supply of substrate for the energy processes and as a backup to NE, if needed.

An interesting characteristic of cold-adapted animals is an increased sensitivity to NE and E. While an increased sensitivity to NE and E is advantageous to the animals, it is contradictory to traditional concepts of the regulation of receptors (201); as cold-exposed animals are characterized by increased circulating levels of the endogenous ligands, NE and E, as already mentioned. An increased sensitivity may occur for several reasons including a) changes in receptor affinity; b) changes post-receptor; for example, either changes in secondary messengers or in the cascade system which follows; and c) decrease in either reuptake or removal of the transmitter. Further, a change in sensitivity in the heat producing systems may imply selectivity in the regulation of receptors for their ligand based on location or function. Since the stimulatory effect of NE on metabolism is related to its beta-adrenergic potential, it might be expected that a similar stimulatory effect on cardiovascular responses, another beta-adrenergic dependent response, would also be observed. In addition, NE also possesses alpha-adrenergic potential. However, Fregly et al. (60) and Bryar et al. (19) have reported a decrease in responsiveness to alpha-adrenergic agonists in aortic rings of cold-adapted rats in vitro. Barney et al. (8) reported an increased metabolic and an unchanged cardiovascular responsiveness to exogenously administered beta-adrenergic agonist, isoproterenol, in cold-adapted rats. Thus, chronic exposure to cold increases the concentration of NE in plasma and the metabolic (beta) responsiveness to exogenously administered NE but actually decreases the vascular (alpha) and the cardiovascular (beta) responsiveness is decreased or unchanged. Thus, the altered responsiveness appears to be related more to function or location rather than to the concentration of the ligand. Whether chronic exposure to cold is unique in this respect is undetermined.

With chronic exposure to cold, the urinary output of NE decreased toward control levels and urinary E output returned to control levels within 30 days (122). This suggests that the concentration of NE in the plasma may decrease toward control levels and E concentrations should not be different from controls after 30 days of exposure. If this is the case, it suggests that the animal is 1) adapted to the generalized stress of cold-exposure, and thus decreasing the concentration of E; 2) meeting substrate demands without additional E release; and 3) increasing its sensitivity to NE, thereby decreasing the need for a greater output of NE. The cold-adapted animal therefore can be characterized by an increased concentration of NE but a normal concentration of E in the plasma, and a specifically enhanced responsiveness to the metabolic effects of these ligands.

Clearly, chronic exposure to cold results in a variety of hormonal, receptor and post-receptor responses, and a large sustained increase in output from the SNS. The majority of work investigating these responses has been directed toward their role in maintaining core temperature and thus survival. Whether these same responses result in additional physiological changes is unknown.

Hypertension

Hypertension is a major risk factor in cardiovascular disease. Cardiovascular disease is not only a major problem among the elderly, it also causes premature deaths with more than 100,000 deaths annually in people under the age of 65 (191). Epidemiological studies, such as the Framingham Study (103) have examined the relationships between cardiovascular disease and many variables, including hypertension. Although not providing direct evidence for cause-and-effect interactions, some important associations are evident between the risk of cardiovascular disease and these predisposing factors.

An elevation of arterial blood pressure is associated with a significantly increased risk of sudden cardiac death, myocardial infarction, and atherosclerosis (71,72,69,191). Further, pharmacological treatment of elevated arterial blood pressure decreases the total cardiovascular morbidity and mortality among hypertensive patients (45). Hence, it is of little wonder that vast amounts of research have been directed toward the mechanisms involved in the elevation of blood pressure during the past decade. In spite of the large amount of work conducted in this area, no single abnormality of any one depressor or pressor system has been demonstrated as the cause of essential hypertension.

Hypertension is a complex and multifactorial syndrome. The syndrome of hypertension is more than just an elevation of arterial pressure, although it is this aspect with which the general public is most familiar. Hypertension when fully developed is characterized by an increase in vascular resistance to blood flow, cardiac hypertrophy, often an increase in cardiac output, an increased output of the SNS, changes within the vascular smooth muscle cells, often atherosclerosis, and abnormalities in renal function. Hypertension, like many other chronic diseases develops silently with small subtle changes occurring in the contributing factors over many years. This, in part, explains why the exact etiology of the hypertension remains unknown. In most cases, the hypertension remains undetected until the most significant symptom of abnormality (i.e. an elevation of arterial pressure) is expressed. It is the increased arterial pressure and resistance to blood flow which are suddenly of great concern.

Increased vascular resistance is due to constriction of the vascular smooth muscle, primarily in the high resistance vessels. Depending on the form of hypertension, various factors are known to alter vasomotor tone, including a) increased activity of the sympathetic nervous system innervating the vascular smooth muscle; b) increased concentration in plasma of vasoactive agents such as NE, arginine vasopressin (AVP), angiotensin II (AII), and the eicosinoids, which stimulate the vascular smooth muscle to constrict; c) alterations in activity within the areas of the central nervous system responsible for regulating the discharge to the periphery via the sympathetic and parasympathetic nervous systems; d) morphological changes in the vascular smooth muscle; and e) reduced renal excretory function due to intrinsic alterations of kidney function or the extrinsic factors mentioned above (a-d). The diversity of these factors, any of which can increase vascular resistance, has resulted in various hypotheses and thus models for hypertension. Due to the unknown etiology of hypertension, the use of various research models, each of which induces the disease by a different mechanism yet with the same end result, is advantageous.

Neurogenic Model

The neurogenic hypothesis and various models stem logically from the ability of the SNS to stimulate vasoconstriction. Sympathetic tone, and thus peripheral vasoconstriction, is controlled by a complex network of neurons located in the ponto-medullary region in the brainstem. The tonic discharge of this center is under negative feedback control (79). Lesions in the nucleus tractus solitarus (NTS) result in excess SNS activity; either a transient or sustained elevation of blood pressure; a loss of the baroreceptor reflex, and a decrease in the plasma concentrations of renin and aldosterone (43). Higher brain centers are also known to influence sympathetic outflow. The hypothalamus-thalamus-septal area has a large number of interneurons connecting it with the NTS. Electrical stimulation of this region results in constriction of resistance vessels, vasodilatation in skeletal muscle, an increase in stroke volume, heart rate, and blood pressure (51). These physiological responses are very similar to those elicited in defense of internal temperature. The NTS region is the first synapse of the baroreceptor pathway. Thus, baroreceptor denervation is the most familiar neurogenic model of hypertension.

In 1958, Heymans and Neil (90) denervated both the carotid sinus and aortic arch of dogs and reported an increased blood pressure. Although the elevation of blood pressure was dramatic in the acute preparation, the pressure was very labile. Utilizing baroreceptor denervated dogs (long-term denervation) and chronically implanted cannulae, Cowley et al. (32) reported no difference in the average level of blood pressure and only an increased variability. These authors concluded that the baroreceptors act to minimize variations in blood pressure rather than to control the absolute level. Ito and Scher (99), as well as others (113,48), disagree. These authors (99,113,48) have demonstrated a small but significant and sustained elevation of blood pressure in dogs and rats upon lesioning of the aortic baroreceptors.

In addition to the direct effects the baroreceptors have on the circulation, neurogenic models may also influence the circulation indirectly via changing AVP, ACTH, or AII levels as well as renal function. The major limitations of the neurogenic model are a) requirement for extensive surgical intervention and b) the lack of evidence demonstrating sustained hypertension rather than increased lability of blood pressure. It is a good model, however, in the respect that central mechanisms which influence blood pressure must certainly be altered in essential hypertension as suggested by the large class of very effective antihypertensive drugs which act centrally on SNS function. In addition, this model permits an investigation of the interaction, or lack of interaction, between the CNS and the kidney.

Pharmacological Model

The most common pharmacologically induced model of hypertension in the rat is that using deoxycorticosterone acetate plus high salt intake (DOCA/salt). This hypertension is salt dependent in its initiation and often involves surgical reduction of renal mass. Thus, the DOCA/salt model is often used in combination with unilateral nephrectomy. This combination results in hypertension, cardiac hypertrophy, and nephrosclerosis (175). The hypertension which develops is thought to be dependent on volume expansion. An increase in fluid volume would increase central venous pressure, cardiac output and thus reflexly lead to vasoconstriction and rarefaction to adjust flow (77,80). In addition, there is evidence that AVP may be important in both the development and maintenance of blood pressure in this model (36,37). The sympathetic nervous system also appears to play a role in the development of DOCA/Salt hypertension (104). The increased output of the SNS may act to change renal function, as denervation of the kidneys affects both the onset and the severity of the disease (104). The major limitations of the DOCA/Salt model are a) the pharmacological doses of drug required; b) requirement for surgical reduction of renal mass; and c) required ingestion of large amounts of NaCl. A significant advantage of this model is the potential to investigate the role of sodium in the developmental stages of hypertension.

Genetic Models

Hypertension and cardiovascular disease (CVD) may have genetic predeterminants. There is a predisposition to hypertension and CVD in those humans with a positive family history. The notion of genetic predisposition for the development of hypertension has been strengthened by the development of genetic or spontaneous models of hypertension. The two most common models are the Dahl salt-sensitive, salt-resistant hypertensive strain and the spontaneously hypertensive strain developed by Okimoto and Aoki.

The spontaneously hypertensive strain (SHR) is used extensively. Some investigators feel it to be the best model for human essential hypertension. Some hemodynamic alterations which occur during the development of the disease are similar to human essential hypertension, i.e. a high cardiac output early, and in the adult, a normal cardiac output and an increased vascular resistance (188). In this model, there is also an increase in the activity of the sympathetic nervous system which may contribute to the development of the disease. Thus, electrical activity in the renal sympathetic nerve is increased (188), while both renal blood flow and glomerular filtration rate are normal (5) and renal vascular resistance is increased (188). The advantage of these similarities with human essential hypertension (29) is important. A major drawback of this model is the lack of an appropriate control, one which has been genetically altered yet is free from the disease and the complexity of the genetic mutations which have affected not only blood pressure but many other regulatory systems as well.

Renal-Dependent Models

One factor which appears consistently altered in all of these models is renal function. That the kidney is involved in sodium and water conservation and, thus, intimately involved in the regulation of blood pressure is well accepted. Since 1934, when Goldblatt et al. (66) induced an elevation of blood pressure by partially constricting the renal artery of the dog, many renal-dependent models have been developed. Basically, this form of experimental hypertension can be divided into two general groups, two-kidney hypertension (clip one kidney while leaving the other intact), and one-kidney hypertension (clip one kidney and remove the other). This form of hypertension has been induced in dogs, rats, rabbits, sheep, and cats (202).

In the two-kidney model, the renin-angiotensin II system has been implicated as a major contributing factor in the pathogenesis of the disease (14,124). The renin-angiotensin II response is biphasic, with an elevation lasting up to 1 week and thereafter returning to normal (14,124). The sustained elevation of blood pressure may still be AII-dependent, however, as Bean et al. (9) have demonstrated the ability of AII to shift its own dose-response curve. Thus, a previously sub-pressor dose of AII would now result in an elevation of blood pressure. The basic hypothesis for the model is that the increase in blood pressure results from the influence of the clipped kidney on the normal kidney. Thus, error signals (changes in hormones and sympathetic nervous system activity) to a normal kidney result in changes in sodium and water handling, and thus, changes in blood pressure.

In the one-kidney model, the early elevation of blood pressure is also AII-mediated (53). Due to the removal of the other normal kidney, no compensatory increase in sodium and water excretion can occur, and hence, fluid volume is retained (18). This model is thus a sodium-fluid volume dependent model.

The hypertension which develops in these two renal-dependent models is reversible, i.e. when the clip is removed, arterial pressure returns to normal (124,125,184). This is a unique feature of these models which has received little attention. Further, if the reversal of hypertension is a time-dependent phenomenon, it would suggest an important window in which changes occur which are not reversible, perhaps structural changes. In addition, these models provide the opportunity to investigate the changes which occur specifically at the level of the kidney, as well as the role of the kidney in long-term blood pressure control.

Summation

Regardless of the model or method of inducing an elevation of blood pressure, the ultimate goal is the development of the hypertensive. Each model has limitations and advantages while none of them clearly duplicates the essential hypertension that occurs in humans. Perhaps the commonalities of an altered activity of the SNS and an alteration in renal function are clues to a unifying mechanism in hypertension. The need for new models that may contribute additional information to an understanding of hypertension is clear. In addition, the investigation of each of these models during the developmental stages of hypertension is imperative in efforts to determine the mechanisms associated with an elevation of blood pressure, and thus effective in treating hypertension.

Cold Hypertension As A Model Of Essential Hypertension.

An interesting characteristic of adaptation to cold in rats, which was originally mentioned in 1950 by Gilson (64), is an elevation of systemic blood pressure. Fregly in 1954 (54) as well as Heroux and Dugal (87) have also reported an increase in systolic blood pressure in the rat, while LeBlanc (118) reported no difference in anesthetized (pentobarbital) rats. An increase in mean arterial pressure in response to acute exposure to cold has also been reported by Thompson et al. (185) in sheep, and in young oxen by Bell and Thompson (10). Since these studies were primarily designed to investigate substrate utilization, metabolism and adaptation during exposure to cold, and since blood pressure measurements were often recorded as secondary information, these observations have received relatively little attention.

Recently however, Papanek and Fregly (151,152) have begun to investigate the effects of chronic exposure to cold on blood pressure in rats. Fregly et al. (61) have measured systolic, diastolic and mean blood pressures in cold-adapted rats with indwelling cannulae. Blood pressure, as well as heart rate and ventricular weight, was significantly elevated in cold-adapted rats (61). The combination of an increase in arterial blood pressure and heart weight led Fregly et al. (61) to conclude that the cold-adapted rat is hypertensive. If this is the case, then chronic exposure to cold represents a unique method for the induction of hypertension which does not involve either surgical intervention or pharmacological dosage of drugs.

Cold-induced elevation of blood pressure is demonstrable in human beings as well as in rats. Reed et al. (162) have reported a significant elevation of mean arterial pressure in young men during extended residence in Antarctica. Over the course of 42 weeks, mean arterial pressure rose from baseline values of 85 mm Hg to 101 ± 3 mm Hg. An elevation of systolic, diastolic and mean arterial pressures in addition to an increase in plasma NE has also been shown in response to acute cold (193,199,172). Scriven et al. (172) showed a strong correlation between the plasma concentration of NE and both systolic and diastolic blood pressures. Further, Brennan et al. (17) have reported seasonal variations in arterial pressure during the British Medical Research Council's treatment trial for mild hypertension. Their results show that for each age, sex, and treatment group, both systolic and diastolic pressures were higher in winter than in summer (17). Likewise, Hata et al. (83) reported a seasonal variation of blood pressure in patients with borderline and established essential hypertension. These authors also reported seasonal variations for plasma NE, urinary catecholamines, and urinary sodium, without variations in plasma renin activity or the concentration of aldosterone in plasma (83). In 1961, Rose (166) reported a seasonal influence on blood pressure during a 1-3 year study of ischemic heart disease. However, his peak values were reported for the spring rather than the winter. With the exception of the study by Rose (166), the above studies suggest a negative correlation of ambient temperature on blood pressures in humans. The study of cold-induced hypertension in rats may allow a better understanding of some of the mechanisms involved in the cold-induced elevation of blood pressure in humans.

CHAPTER TWO
COLD-INDUCED HYPERTENSION IN RATS

Introduction

Exposure of either rats or humans to low environmental temperatures results in a variety of physiological responses aimed at maintaining body temperature at or near normal. The remarkable constancy of core temperature in the face of varying environmental temperatures received attention early in physiological studies. Cannon noted in "The Wisdom of the Body" that temperature was so well maintained from individual to individual, that manufacturers actually stamped "normal" at 98.6°F on their thermometers (23).

Initial responses to sudden exposure to low environmental temperature are a reduction in heat loss (piloerection and peripheral vasoconstriction), and an increase in heat production. Initially, the increase in heat production is accomplished neuromuscularly, i.e. by shivering. As exposure to cold continues, the emphasis is shifted away from neuromuscular mechanisms and toward biochemical mechanisms, i.e. nonshivering thermogenesis. Much of the work investigating cold has centered on the hormonal and metabolic factors involved in thermogenesis, and in adaptation to cold (57,59,92,141). Cold-adapted animals are characterized by an increase in nonshivering thermogenesis (NSTG) which is attributed both to changes in heat producing mechanisms (88) and to a supersensitivity to norepinephrine (NE) (41,96). An increased sensitivity to NE by heat producing systems is interesting because it occurs in the presence of an increased concentration of circulating levels of the endogenous ligands, NE and epinephrine (E) (121,122). Leduc (122), Lutherer et al. (133) and many others (100,183,6) have measured large increases in urinary outputs of NE and E during chronic exposure to cold. An increased metabolic sensitivity to NE is advantageous to the cold-exposed animal, and suggests a change in the regulation of receptors induced by exposure to cold. However, this suggestion is contradictory to the traditional concepts of the regulation of receptors (201).

An important characteristic of exposure to cold in rats, which was originally mentioned in 1950 by Gilson (64), is an elevation of systemic blood pressure. Similarly, Fregly (54) in 1954, as well as Heroux and Dugal (87) have also reported an increase in systolic blood pressures in the rat, while LeBlanc (118) reported no difference in blood pressure. However, in the experiment by LeBlanc (118), the rats had been anesthetized with pentobarbital, heparinized, and removed from the cold. The depressant effects of pentobarbital on blood pressure are well known. An increase in mean arterial pressure in response to acute exposure to cold has also been reported by Thompson et al. (185) in sheep, and in young oxen by Bell and Thompson (10).

With the exception of the work of LeBlanc (118), the majority of the studies using rats were designed to investigate either substrate utilization or other adaptive mechanisms during exposure to cold, and blood pressure measurements were recorded for the most part as secondary information. The observation that chronic exposure to cold resulted in the development of a sustained elevation of blood pressure has received relatively little attention. Recently however, Fregly et al. (61) measured systolic, diastolic, and mean blood pressures in cold-adapted rats with indwelling cannulae. Blood pressure as well as heart rate and heart weight were significantly elevated in rats exposed to cold (6°C) for 4 weeks. The combination of an increase in blood (systolic, diastolic, and mean) pressure and heart weight led Fregly et al. (61) to conclude that the cold-adapted rat is hypertensive. If this is the case, then cold-adaptation represents a unique method for the induction of hypertension which does not involve either surgical intervention or pharmacological dosage of drugs.

The purpose of the first series of experiments was to describe the development of hypertension in rats chronically exposed to air at 6°C.

Methods

Male rats of the Sprague Dawley strain initially weighing 200-250 g were used. All animals were housed in individual cages and provided powdered Purina Laboratory Chow (#5001) and tap water ad libitum. The vivarium was maintained at 26°C and on a 12:12 light:dark cycle. Two weeks were allowed for the animals to adapt to their new environment.

Experiment One

Twelve male rats were divided randomly into two equal groups, i.e. either a control (26°C) or a cold-treated (6 ± 2°C) group. Body weights and systolic blood pressures (SBP) were measured during the initial two week control period with all animals maintained at 26°C. At 0800 h on the first experimental day, all animals were moved into temperature controlled environmental chambers (either 26 or 6°C). Systolic blood pressure and body weight (BW) were recorded weekly throughout the remainder of the experiment.

Systolic blood pressures were measured from unanesthetized rats indirectly from the tail. Animals were removed from their home environments and placed into stainless steel restraining cages. The restrained animals were placed onto a heating pad and were warmed by means of a heat lamp. Ambient temperature was maintained at 30 ± 2°C. Approximately 15 minutes were allowed for the animals to vasodilate prior to beginning pressure measurements. Systolic blood pressure was measured using a pneumatic pulse transducer (MK III series), coupled to a Narco Bio (model DMP-48) physiograph recorder. The occlusion cuff was always placed at the base of the tail to insure reproducibility. This procedure for the measurement of SBP is essentially that previously described by Fregly (56). The SBP data represent the mean of ten measurements per animal. At the end of the measurements, the animals were immediately returned to their home environments.

Experiment Two

Twenty male rats were divided equally into either a control or cold-treated group. Each animal was provided with a spill-resistant food container (55). Fluid containers consisted of infant nursing bottles with cast bronze spouts (117). These minimized, but did not eliminate spillage. Reservoirs were attached under each water bottle to collect any water that spilled. Each cage was fitted with a stainless steel funnel for the collection of urine separate from feces. Urine was collected into volumetric tubes. Food and water intakes were determined gravimetrically. Water intakes were corrected for spillage. Baseline measurements were recorded for two weeks. On the first experimental day, all animals were moved into temperature-controlled environmental chambers as described above. All measurements were recorded daily at 0800 h throughout the duration of the experiment.

At the end of each experiment, animals were sacrificed and the heart, kidneys, adrenal and thyroid glands were rapidly removed. All organs were trimmed of fat and connective tissue, blotted and weighed on a torsion balance. The heart was then subdivided further: the atria were removed first, then the right ventricle was lifted and cut away leaving the left ventricle and the entire septum. The atria, right and left ventricles were weighed. In experiment one, the interscapular brown fat and epididymal white fat were removed and weighed. After the initial weights were recorded, the organs were dried overnight in an oven (Boekel, 65°C) to remove all water and reweighed. Results are expressed as mg tissue/100 g of body weight. Microhematocrit tubes were filled in duplicate at the time of sacrifice in experiment two and immediately centrifuged for four minutes in a microcapillary centrifuge (Model MB, International Equipment Corp.).

Statistical analyses of SBP and BW were carried out by a two-way analysis of variance repeated in time. Differences in organ weights were determined by a one-tailed, Student's t-test for independent samples. The hypothesis tested was that the variable being tested in cold-treated animals was less than or equal to that of controls

(H_0: cold $\leq$ control, while H_a: cold > control). Significance was set at the 95% confidence interval. A posteriori pairwise comparisons were performed when appropriate by a Duncan's New Multiple Range test which maintained the significance at the a priori level ($p < 0.05$) (111).

Results

Chronic exposure to cold resulted in a significant elevation of systolic blood pressure (Figure 2-1). Throughout the initial exposure to cold (2 weeks), no change in systolic blood pressure was detected (Figure 2-2). The first significant elevation of systolic blood pressure occurred after 21 days of exposure to cold. The systolic blood pressure of the cold-treated group remained significantly elevated for the duration of the study. In each experiment, the body weights did not differ significantly between the two groups prior to treatment (cold). However, there was a significant interaction between environmental temperature and time. The slope of the line of body weight versus time is depressed; i.e. cold-exposed rats gained weight at a slower rate (Figure 2-3, 2-4). In addition, the cold-exposed group had a significantly increased amount of interscapular brown adipose tissue (IBAT) and a decreased amount of white adipose tissue (Table 2-1). These are characteristic changes associated with acclimation to cold. Due to differences in body weights, data for the intakes and outputs were expressed per unit of body weight (ml or g/100 g BW). Water and food intakes, as well as urine outputs, of the two groups were similar during the control period (Table 2-2). Food and water consumption increased significantly in the cold-exposed animals as demonstrated by both a significant interaction and a main effect (Figure 2-5, 2-6). Both food and water intakes peaked after one week. Daily urine output increased significantly as well and followed the increase in water intake (Figure 2-7). These results are similar to those previously reported by Fregly et al. (58).

A regression analysis of food versus water intake indicated that the cold-exposed group drank less water for any given food intake (Figure 2-8). Regression analysis of

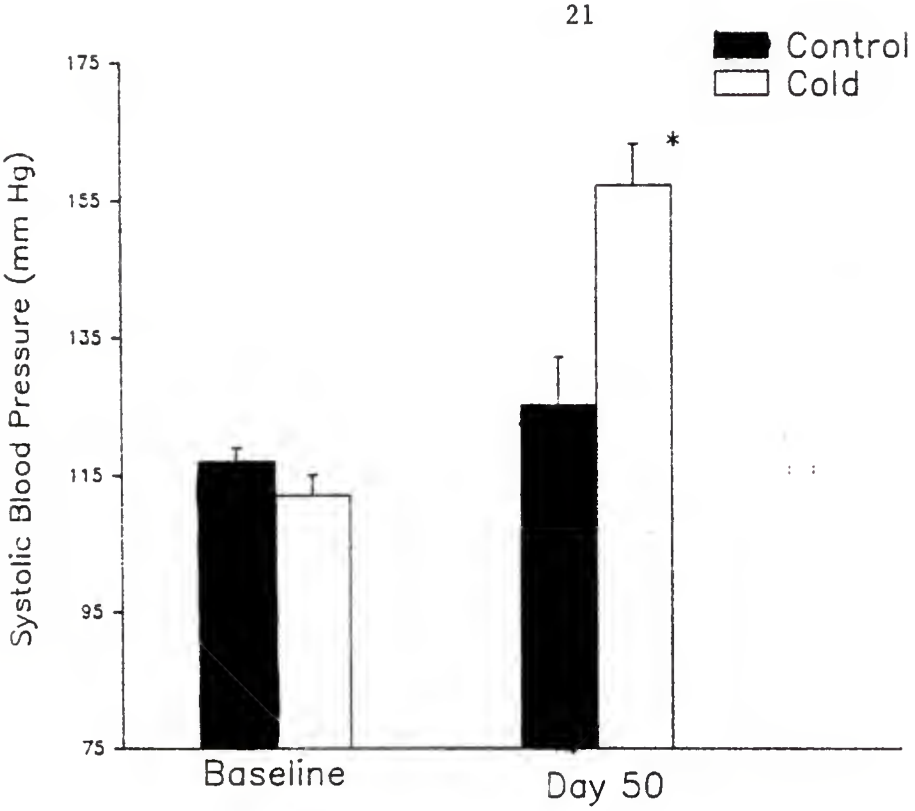
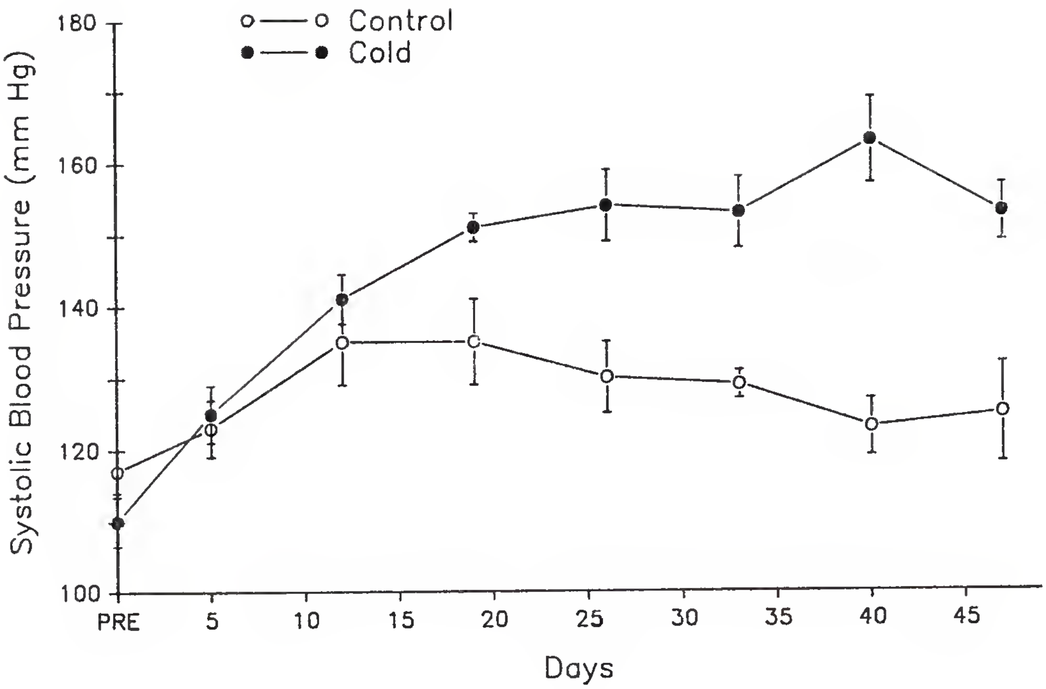
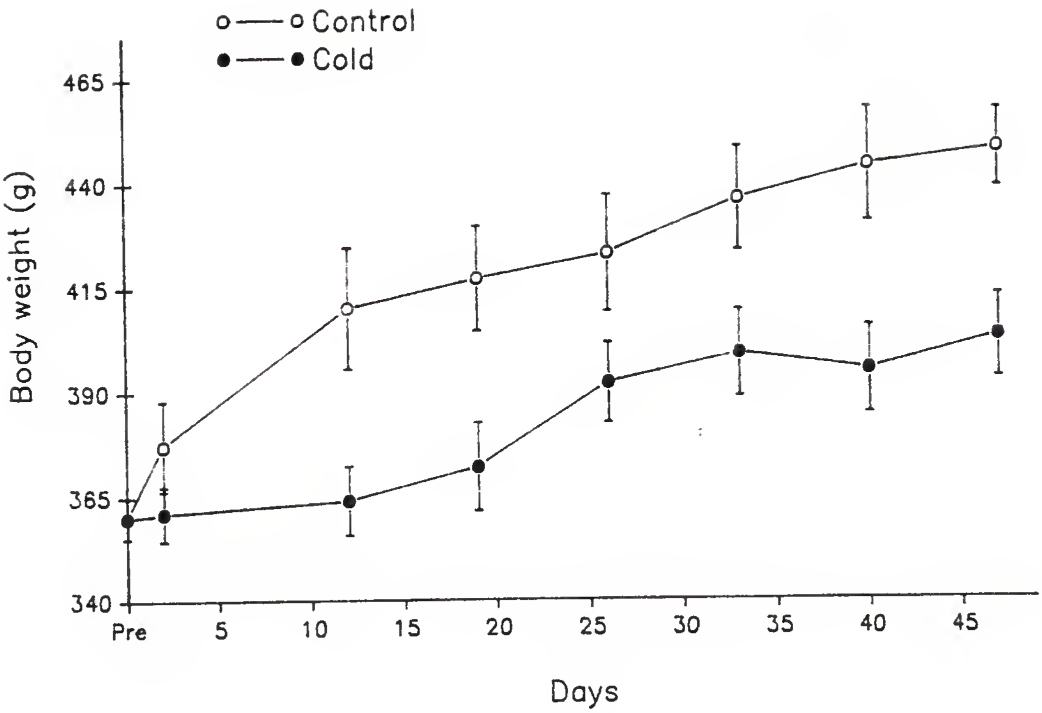


Figure 2-1. The effect of 50 days of exposure to cold on systolic blood pressure of rats.
Values are Mean ± S.E.M.
* Significantly greater ($p < 0.05$) than control by one-tailed, independent-sample, t-test.



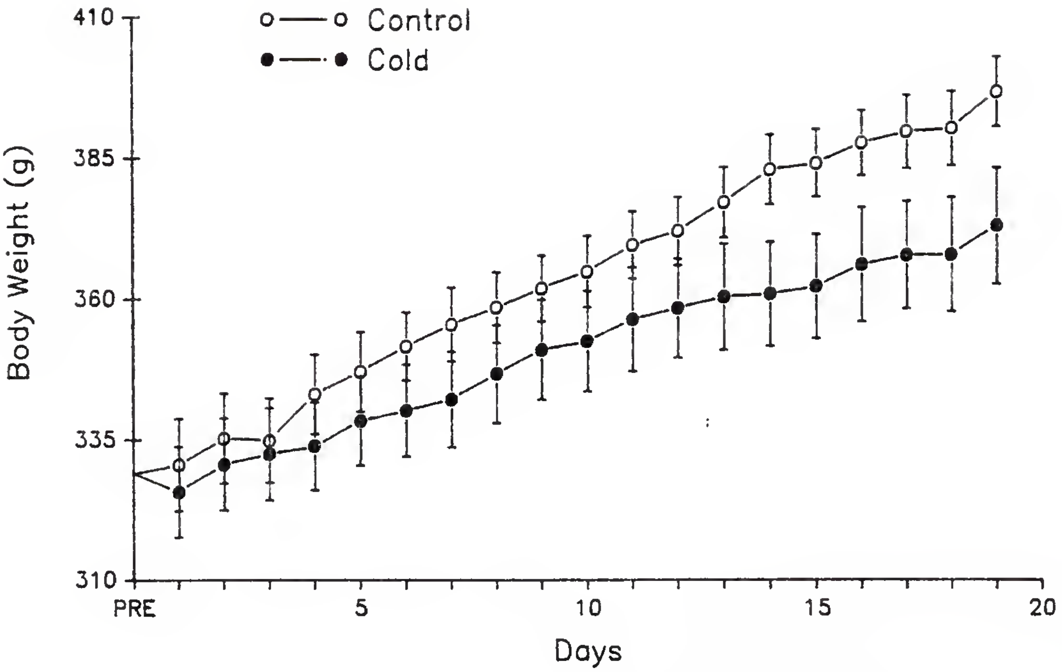
Source	SS	DF	MS	F	P
BETWEEN Environment	5386.9	1	5386.9	11.62	<0.01
Error	5099.3	11	463.6		
WITHIN Time	19819.9	8	2477.5	14.91	<0.01
Env. * Time	7323.5	8	915.4	5.5	<0.01
Error	14626.6	88	166.2		

Figure 2-2. The effect of 47 days of exposure to cold on systolic blood pressure of rats.



Source	SS	DF	MS	F	P
BETWEEN Environment	38642.9	1	38642.9	9.9	<0.01
Error	43080.7	11	3916.4		
WITHIN Time	37291.0	7	527.3	50.8	<0.01
Env. * Time	2703.7	7	86.2	3.7	<0.01
Error	8078.3	77	104.9		

Figure 2-3. The effect of chronic exposure to cold on body weight of rats. Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN					
Envirn.	16245	1	16245	1.47	.241
Error	188388	17	11081		
WITHIN					
Time	119224	19	6275	253.4	<0.01
Env.*Time	5418	19	285.2	11.51	<0.01
Error	7999.7	323	24.8		

Figure 2-4. The effect of chronic exposure to cold on body weight of rats from experiment two.
Values are Mean ± S.E.M.

Table 2-1. The effect of chronic exposure to cold on the ratio of organ to body weight of certain organs of the rat.

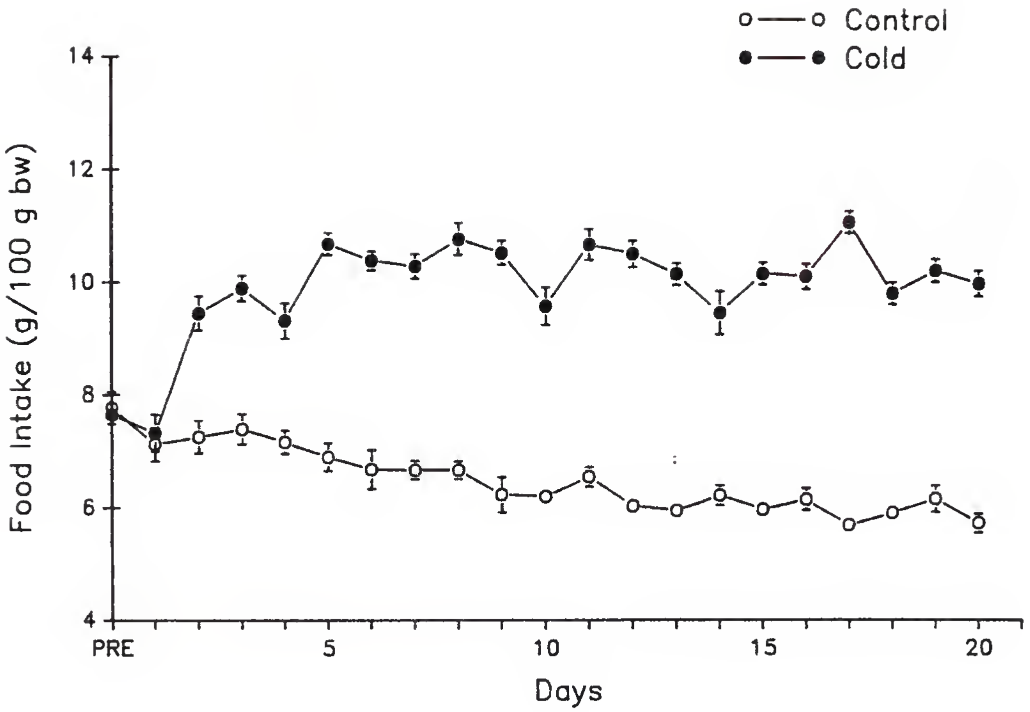
	Organ to body weight ratio (mg/100 g) of:									
	Heart	LV	RV	Atria	Kidneys	Adrenals	Thyroid	IBAT	White Fat	Final BW
COLD										
20 days	329.0±3.8*	222.0±3.2*	62.0±1.7*	28.0±1.3*	956.0±25.0*	17.0±0.7*	4.9±0.3	nd	nd	376.0±10.0
52 days	nd	238.0±5.4*	80.2±4.1*	30.6±1.1	881.6±31.8*	21.9±0.9*	nd	191.0±10.2*	108.1±8.2t	405.0±9.5
CONTROL										
20 days	285.0±6.3	191.0±6.0	53.0±1.5	22.0±0.95	738.0±23.0	12.5±0.5	4.4±0.3	nd	nd	397.0±6.1
52 days	nd	186.1±8.8	58.0±5.3	31.6±5.9	707.8±38.0	16.3±2.0	nd	50.0±4.1	118.0±12.0	446.2±11.3

Values are Mean ± S.E.M.
* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.
t Significantly less than control ($p<0.05$) by independent-sample, one-tailed t-test.
nd = not determined.
IBAT: interscapular brown adipose tissue.
white fat: epididymal fat pad.

Table 2-2. The effect of 20 days of exposure to cold on daily intakes of food and water, and urine output.

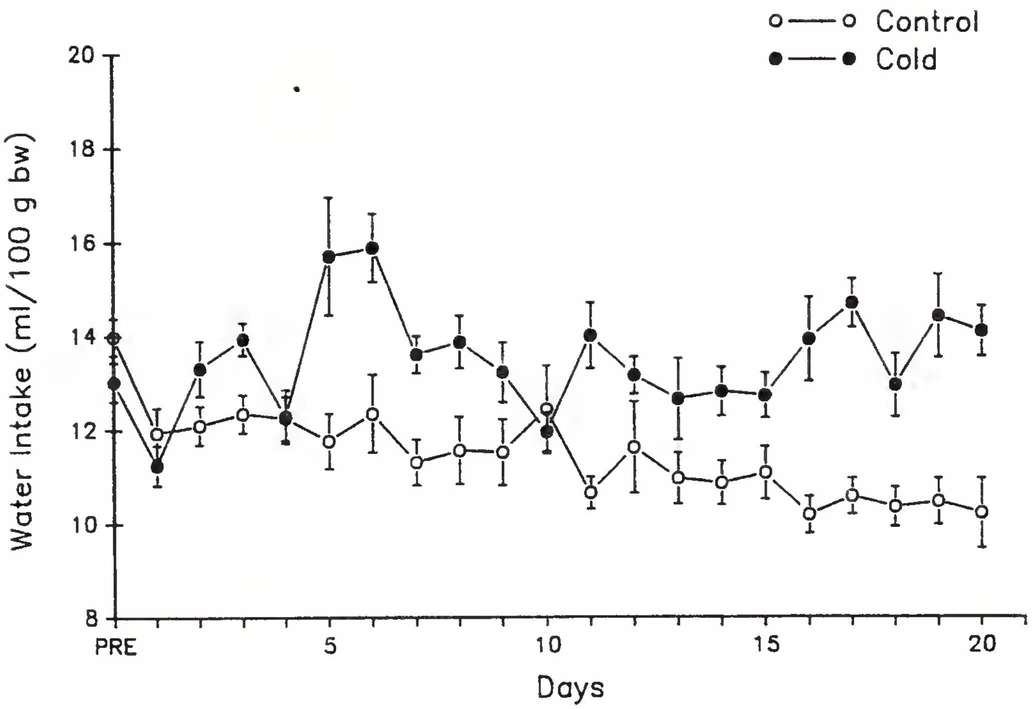
	BASELINE			FINAL WEEK		
	Food	Water	Urine Volume	Food	Water	Urine Volume
COLD	7.7 ±0.13	13.4 ±0.53	4.13 ±0.38	10.03 ±0.2	14.7 ±0.62	7.67 ±0.51
CONTROL	7.8 ±0.29	14.0 ±0.4	4.52 ±0.26	5.9 ±0.1	10.4 ±0.56	4.08 ±0.39

Values are Mean ± S.E.M. and represent a three-day average.
Food is expressed as g/100 g BW.
Water and Urine volume are expressed as ml/100 g BW.



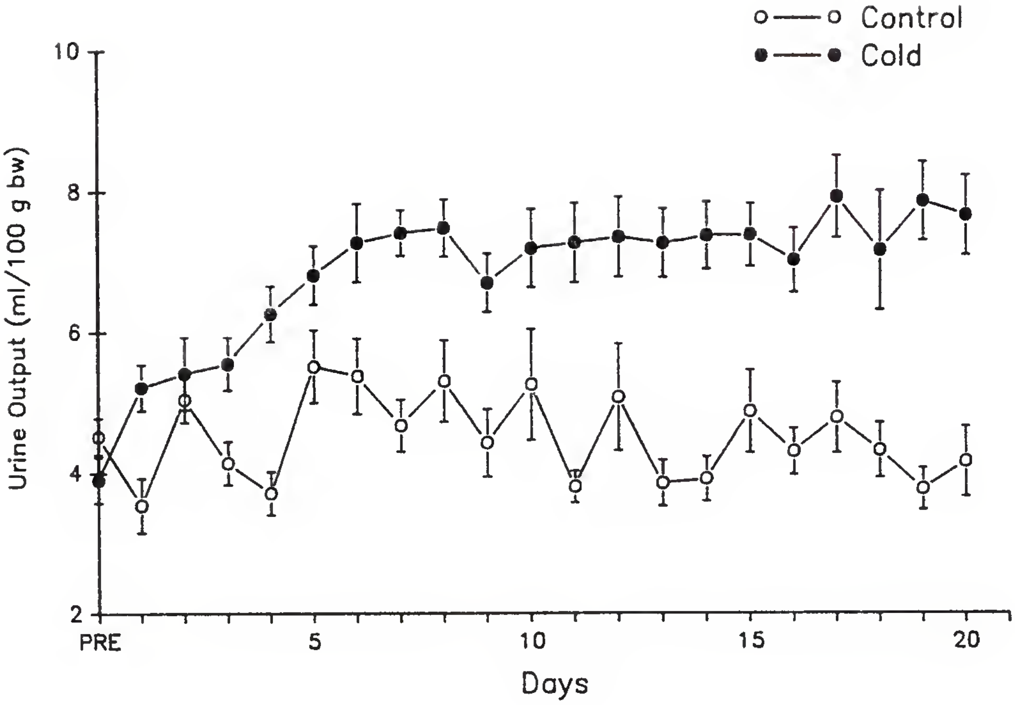
Source	SS	DF	MS	F	P
BETWEEN					
Envirn	1151.76	1	1151.76	329.26	<0.01
Error	59.47	17	3.5		
WITHIN					
Time	56.23	20	2.81	7.62	<0.01
Env.* Time	173.15	20	8.65	23.45	<0.01
Error	125.3	340	0.37		

Figure 2-5. The effect of chronic exposure to cold on the daily intake of food of rats.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN					
Envirn	8965.8	1	8965.8	5.56	<0.05
Error	29031.1	18	1612.8		
WITHIN					
Time	3984.5	20	199.2	4.28	<0.01
Env.*Time	3866.6	20	193.3	4.15	<0.01
Error	16757.9	360	46.6		

Figure 2-6. The effect of chronic exposure to cold on the daily intake of water of rats.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN					
Environment	613.9	1	613.9	23.3	<0.01
Error	474.4	18	26.4		
WITHIN					
Time	164.9	20	8.5	9.8	<0.01
Env.*Time	125.8	20	6.3	7.5	<0.01
Error	303.7	360	.84		

Figure 2-7. The effect of chronic exposure to cold on the daily output of urine of rats.
Values are Mean ± S.E.M.

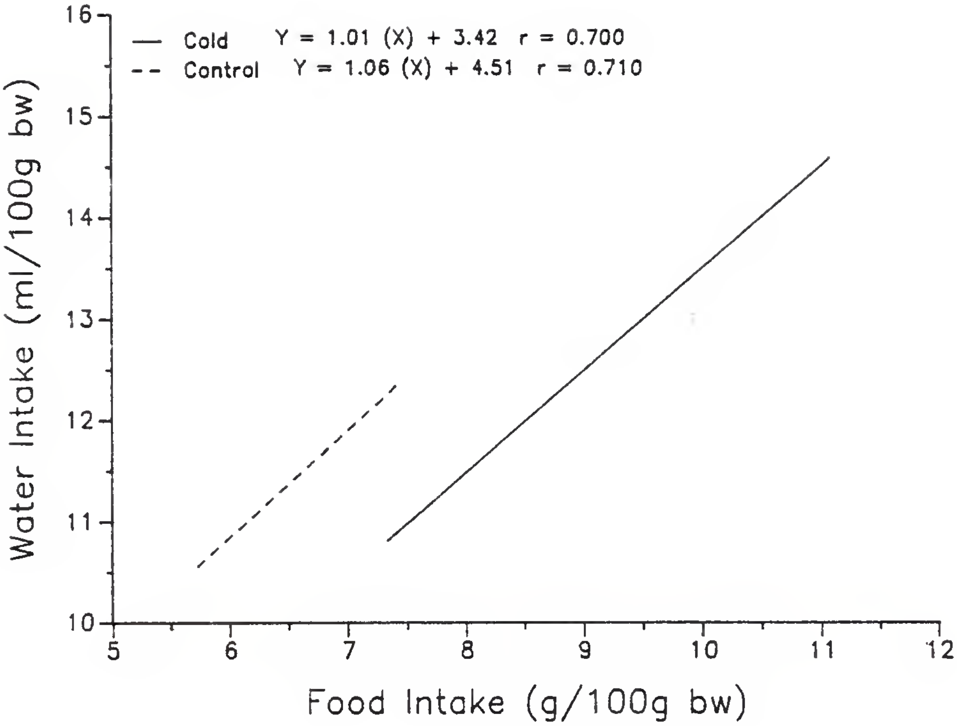


Figure 2-8. The effect of 21 days of exposure to cold on the relationship between the intake of food and water as determined by linear regression analysis.

water intake versus urine output revealed that the cold-exposed group excreted more urine for any given amount of water intake (Figure 2-9).

The effect of chronic exposure to cold (20 and 52 days) on organ weights is presented in Table 2-1. Exposure to cold for 52 days significantly increased the weight of the adrenal glands, kidneys, atria, left and right ventricles as well as IBAT. Exposure to cold for 21 days resulted in significantly increased weight of the heart, kidneys, atria, left and right ventricles and the adrenals. No change in the weight of the thyroid was found in either experiment. In order to determine whether an increase in fluid retention may have contributed to the increased weight of the heart and kidneys found in Experiment 1, the atria, ventricles and kidneys from Experiment 2 were weighed, then dried overnight in an oven and reweighed. The atria, left ventricle, and kidneys of the cold-exposed group remained significantly greater than controls (Table 2-3).

Discussion

Although an earlier study by Fregly et al. (61) showed that systolic, diastolic, and mean blood pressures of cold-exposed rats were elevated after 4 or more weeks of exposure to cold, no information was available regarding the time required for the first elevation of blood pressure to occur. The results from the present study confirm that of Fregly et al. (61) that chronic exposure to cold results in a significant elevation of systolic blood pressure. The present studies indicate that the first significant elevation of blood pressure occurred within 21 days of exposure; i.e. about one week earlier than previously demonstrated. Systolic blood pressure remained elevated throughout the duration of exposure. The development of cold-induced hypertension can be divided into three rather distinct time-periods (Figure 2-2). Period one is characterized by the many physiological changes which permit the animal to survive in the cold. During this time, no change in blood pressure was observed. Period two is the transition period. Within a rather narrow interval of time (18-25 days), all of the cold-exposed animals became hypertensive. Typically, a few animals made the transition early (15-21 days), while a portion became hypertensive up to a week later (21-28 days). In every instance, all of

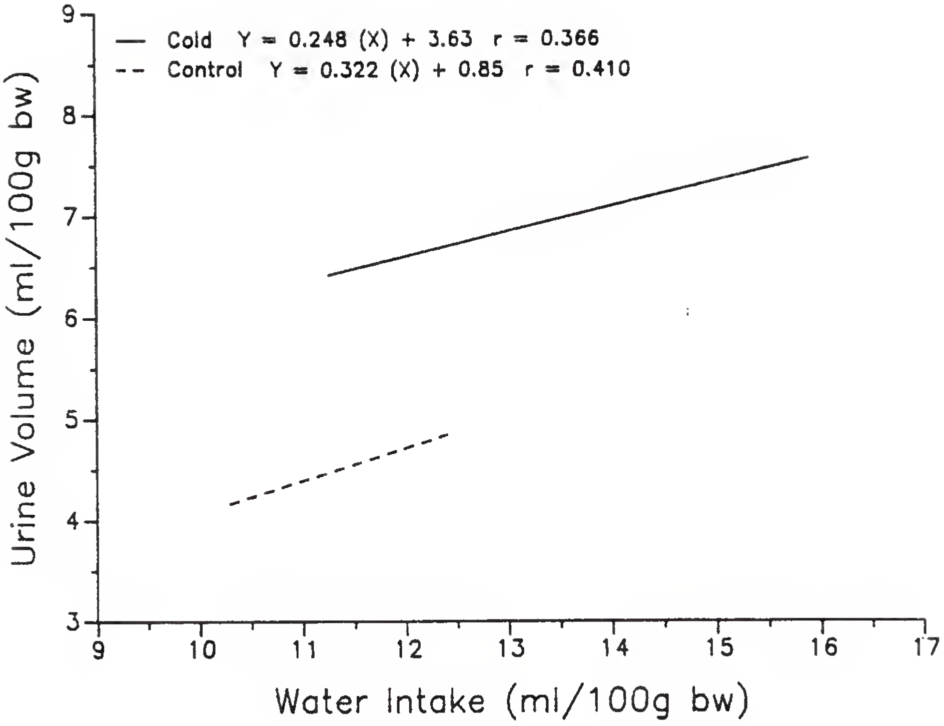


Figure 2-9. The effect of 21 days of exposure to cold on the relationship between the daily intake of water and the output of urine as determined by linear regression analysis.

Table 2-3. The effect of 20 days of exposure to cold on the weight of certain desiccated organs of the rat.

	Left Ventricle (mg)	Right Ventricle (mg)	Atria (mg)	Kidneys (mg)
COLD	206.5 ± 4.1*	60.0 ± 1.4	22.6 ± 1.3*	878.0 ± 35.0*
CONTROL	194.1 ± 5.9	57.4 ± 1.5	19.9 ± 0.7	717.5 ± 11.8

Values are Mean ± S.E.M.
* Significantly greater than ($p<0.05$) control by independent-sample, one-tailed t-test.

the animals were hypertensive in these two experiments by the 25th day of exposure. Period three is the period of maintenance. The animals have fully adapted to the cold but have become hypertensive. Although these experiments continued for only 52 days, others have exposed rats to low environmental temperature for as much as three months (122), or longer, (22 months) (95). This suggests that chronic exposure to low temperature is well tolerated by the rat. In addition, it provides evidence that although chronic exposure to cold represents an unremitting stress, it is different from other general stressors. Holloszy and Smith (95) reported that chronic exposure to cold was not associated with the deleterious effects on health which have been shown for other stressors such as fear, noise, or overcrowding (86,153,167).

It is well known that one of the major mechanisms by which the cold-exposed rat increases heat production is by an increase in nonshivering thermogenesis and a supersensitivity to norepinephrine (NE). Exposure to cold results in a large increase in sympathetic activity. Initially, the response may be characterized as a generalized "stress" response similar to that described by Selye (174). This is evident by a large increase in adrenal cortical and medullary hormones, i.e. glucocorticoids and epinephrine (E), respectively (158,192). In addition, there is a large increase in urinary concentrations of NE and E during acute exposure to cold (133,183,122). Leduc (122) measured urinary catecholamines in rats exposed to 3°C for up to 86 days. The concentration of NE in the urine increased threefold, peaked within the first week of exposure, and continued to decline thereafter until the study was concluded. After 86 days, urinary output of NE remained two times greater than controls while the concentration of E in the urine had returned to control levels within 30 days.

Norepinephrine is the neurotransmitter of the sympathetic nervous system (SNS). The increased urinary levels of NE suggest that the activity of the SNS is significantly elevated by both acute and chronic exposure to cold. In humans, acute exposure to cold (cold-pressor test) is often used as an index of SNS reactivity. The cold-pressor response is characterized by an increase in blood pressure, heart rate, NE and E concentrations in plasma (199,193). An increase in sympathetic output to the periphery will induce vasoconstriction, decrease blood flow, and thereby decrease peripheral heat loss. This is a beneficial and well documented physiological response to cold. The decrease in flow is accomplished by an increase in resistance in the peripheral vasculature. Thus, an increase in peripheral resistance should result in an elevation of arterial pressure. This is the case in the cold-pressor test mentioned above. The cold-pressor test increases the concentration of catecholamines in plasma and results in an increase in blood pressure. In the current studies, chronic exposure to cold did not result in an immediate elevation of blood pressure (during period one). Blood pressure did not increase until nearly 21 days of exposure. In view of this, the results suggest that any initial increase in peripheral resistance in the cold-exposed rat was compensated for elsewhere. What compensations may actually occur is unknown, but may involve changes in either blood volume, cardiac output, or resistance in other vascular beds.

Hypertension is induced primarily by an increase in vascular resistance to blood flow. As a consequence, blood pressure increases to maintain the proper flow of blood to tissues and cells. The heart, which must pump against a higher peripheral vascular resistance undergoes work-induced hypertrophy. In both of these studies, as in numerous other reports, chronic exposure to cold resulted in significant hypertrophy of the heart. Additionally, in the present studies, there was an increase in left ventricular weight of both of the cold-treated groups. Ventricular hypertrophy was evident after 21 days of exposure, the earliest these measurements were made. These results indicate that cardiac hypertrophy occurred either at the same time, or in some animals, just prior to a measurable increase in systolic blood pressure. There are several possible explanations for the cardiac hypertrophy demonstrated in this model. The most likely is an increased workload on the heart due to an increased total peripheral resistance associated with the development of hypertension. Certainly this is an important factor in those animals which demonstrated an elevation of blood pressure. An additional explanation is suggested by the results of the intake study. The cold-exposed animals drank less water for any given amount of food consumed. Further, these animals excreted more urine for any given amount of water consumed. These results suggest that the cold-exposed animal is in a state of self-imposed, or voluntary volume depletion or contraction. That the cold-exposed rat is volume contracted has been suggested previously by Fregly et al. (58). Whether this altered fluid balance is an attempt by the animal to compensate for the increased central blood volume which would result from increased peripheral resistance is speculative. If the animal is volume contracted, an increased hematocrit and an increased viscosity would likely result. An increased viscosity could also impose a greater work load on the whole heart and could contribute to the generalized cardiac (atria, right and left ventricle) hypertrophy seen in this model. The effect of viscosity on blood pressure is often overlooked, but in accordance with Hagen-Poiseuille's Law, peripheral vascular resistance is dependent on the viscosity of the blood. A significantly increased hematocrit was found in the group exposed to cold for 21 days (49 ± 0.86 and 47 ± 0.4 for controls). Similarly, a significantly increased hematocrit was found in elderly hypertensive patients by Tibblin et al. (186). An increase in serum osmolality after exposure to cold has been reported previously by Fregly et al. (58).

A final contributing factor to cardiac hypertrophy may be the increased sympathetic drive to the heart. Both exercise training and long term exposure to cold result in an increased SNS activity, an increased cardiac output, and cardiac hypertrophy. That the SNS activity is important in inducing this hypertrophy has been demonstrated by chronic injections of NE (81), E (47), and the specific B-adrenoceptor agonist, isoproterenol (147), all of which result in cardiac hypertrophy. Further, a sympathetic blockade with guanethidine prevented the development of cardiac hypertrophy in both exercise-trained and chronic isoproterenol-treated rats (147). In the case of chronic exposure to cold, the increased activity of the SNS could explain the development of cardiac hypertrophy without a significant elevation of blood pressure when other compensatory mechanisms have been invoked.

The dependence of cardiac hypertrophy on excess adrenergic stimulation could be demonstrated in this model by chronic treatment with a beta adrenoceptor antagonist or even guanethidine as used above. However, nonshivering thermogenesis is likewise a beta-adrenergic dependent system. Future studies will be needed to assess the role of the adrenergic system in both the cardiac hypertrophy and hypertension seen in this model.

An increase in right ventricular weight is an interesting finding. It is tempting to suggest that the cold air results in a localized vasoconstriction within the pulmonary circulation. Indeed, pulmonary vasoconstriction does contribute to the right ventricular hypertrophy and hypoxia-related hypertension seen commonly in cattle at high altitude, i.e. Brisketts Disease. However, when Mather et al. (137) compared pulmonary artery, left atria and core temperatures in dogs exposed to extreme cold (-18°C), no differences (gradient $< 0.01^{\circ}C$) were found between the pulmonary artery and the left atria. Over time, all three temperatures fell, suggesting an overwhelmingly low temperature, but no differences occurred between temperatures in the pulmonary artery and left atria. Thus, it appears unlikely that pulmonary vasoconstriction in response to breathing cold air contributes to right ventricular hypertrophy seen in the present studies. A more logical hypothesis is an overall cardiac hypertrophy induced by the high cardiac output necessitated by exposure to cold.

Significant hypertrophy of the kidneys and adrenal glands was also seen in response to cold. These findings are in accord with the work of many others. No doubt the hypertrophy seen in the kidneys is due primarily to the very large increase in food ingested by the cold-treated group. Hypertrophied adrenal glands suggest that the animals have not, as yet, adapted to the stress of cold air. This is in contrast to the concentration of epinephrine in the urine which is no longer elevated at 30 days, suggesting that adaptation has occurred. Reasons for this apparent conflict are unclear.

The present studies, in combination with the previous work of others, raise some intriguing questions about cold-induced hypertension. When the rat is exposed to cold, there is an immediate and apparently sustained increase in the activity of the SNS. That this occurs is supported by an increased urinary NE (133,121,122,147), increased plasma NE and an increased cardiac output (89). Initial indications would suggest cold-induced hypertension to be the neurogenic model of hypertension which investigators have been seeking. If this is the case, why then doesn't the rat immediately become hypertensive and remain as such? What mechanisms are effective in initially offsetting this increased activity of the SNS? Why are they ineffective later? In this respect, cold-induced hypertension appears similar to other models of hypertension (DOCA/salt and renal encapsulation) in that it takes about 3 weeks for the hypertension to develop. If increased activity of the SNS is involved, this slow onset may suggest either structural changes or damage at the level of the kidney or vasculature due to altered function induced by continued high stimulation.

One possible mechanism which may result in changes in peripheral resistance over time would be changes in the vascular reactivity to stimulation by the SNS. Fregly et al. (61) tested the vascular responsiveness to an acute injection of both alpha- and beta-adrenoceptor agonists in cold-induced hypertensive rats. These measurements were made in unanesthetized, unrestrained rats which remained in the cold. The authors found a decreased alpha-adrenergic or vasoconstrictor response and an unchanged, or possibly reduced, beta-adrenergic (vasodilator) response. Similar results for alpha-adrenoceptor stimulation were found by Bryar et al. (19) in aortic smooth muscle and by this author (unpublished results) in femoral artery smooth muscle of cold-acclimated rats in vitro. However, the response to the beta-adrenoceptor agonist, isoproterenol, suggested a greater vasodilator sensitivity in isolated smooth muscle in vitro. These changes would appear to be protective mechanisms induced by cold which would work against a more severe elevation of blood pressure. Whether these changes occur early in the adaptation process is unknown.

As demonstrated by the significantly increased (60% over controls) food intakes, it can be expected that the intake of sodium is similarly increased. Whether an increase in sodium, and thus a volume expansion, occurs and contributes to the development of hypertension is undetermined. However, the results from the present studies as well as those of an earlier study by Fregly et al. (58) suggest that the cold-exposed rat is actually in a state of voluntary dehydration or volume contraction. At any given food intake the water intake is lower; further, for any amount of water ingested, the amount of urine excreted is greater in the cold-exposed rat. Once the rat is removed from the cold, a large post-cold drink is demonstrated. In addition, the increased hematocrit seen in the present study supports the idea of volume contraction rather than volume expansion in this model. If the animal is volume contracted, it may represent a mechanism which was called upon to compensate effectively for the initial vasoconstriction induced by the immediate increase in the output of the SNS. Whether this occurs remains to be tested. Further, whether the excessive sodium intake which occurs due to increased intake of food plays a role in this model of hypertension remains to be considered.

Finally, whether changes in either the baroreceptor system or in the circulating levels of the vasoactive hormones occur and contribute to this form of hypertension also remains for further investigation.

CHAPTER THREE
THE EFFECT OF A LOW SODIUM DIET ON COLD-INDUCED HYPERTENSION

Introduction

The importance of fluid volume in the regulation of arterial pressure has been accepted for a great many years. A complex interaction or link between fluid volume homeostasis and regulation of blood pressure is involved in the control of the excretion of water and sodium by the kidneys. Neural, hormonal and intrinsic renal mechanisms appear to control the excretion of water and sodium and thus, arterial pressure. The basic idea that the kidneys act as a final and overriding regulator of blood pressure is based on a simple feedback loop. When arterial pressure is elevated, an increase in sodium and water excretion occurs which decreases blood volume (pressure-natriuresis), and thus returns blood pressure to control levels. This suggests that an increase in the excretion of sodium is a protective mechanism against a sustained elevation of blood pressure. Conversely, this relationship suggests that an increase in the intake and/or a retention of sodium may contribute to the development of hypertension if the sodium were not completely removed.

Epidemiological and clinical studies suggest a relationship between the intake of sodium and the incidence of hypertensive. For example, Dahl (38) examined this relationship in several distinct populations. At a low intake, Eskimos from Alaska had no incidence of hypertension. Conversely, at the other extreme, the prevalence of hypertension was 39% of the population for men and women from Northern Japan whose average intake of salt was 26 g/day. Further support for the role of sodium in hypertension stems from the use of sodium restriction and/or the administration of a diuretic as a major antihypertensive therapy. In addition, several experimental models exist which are dependent on an increased consumption of salt; for example, the Dahl salt-sensitive and DOCA/salt models. Additional evidence stems from the classical experiment of Meneely et al. (138) in which rats were placed on high sodium chloride intakes and hypertension developed. Further, Weinberger et al. (197) studied the effects of altered sodium intake in normotensive young men and demonstrated a significantly increased blood pressure with dietary sodium loading.

In the first series of experiments investigating cold-induced hypertension, a large increase in food consumption was demonstrated (Chapter Two). An increase in food intake is required by the animal in order to meet the increased energy demand required for an elevation of metabolic rate. The cold-exposed animal increased its intake of food by 50 to 60%. As a result of this increase, the animal also ingested 50 to 60% more sodium than controls. Thus, the animal is forced by the need for increased calories used for heat production to increase the intake of food and hence sodium. The purpose of these experiments was to determine whether the increase in the intake of sodium by animals chronically exposed to cold contributes to the development of cold-induced hypertension.

Methods

Experiment One

Male rats of the Sprague Dawley strain used in this experiment were the same animals used in Experiment 2, Chapter Two. All animals were housed and handled as described previously. After the urine volume was recorded, an aliquot was removed for the determination of the concentrations of sodium and potassium by flame photometry (Technicon Autoanalyzer). In addition, an aliquot of urine was removed for the determination of refractive index. A hand-held refractometer (American Optical, Buffalo, NY) was used for these measurements. Baseline measurements were recorded for two weeks. As described previously, on the first experimental day, all animals were moved into temperature controlled environmental chambers (26 or 6°C). Measurements were recorded daily at 0800 h throughout the duration of the experiment. Rats were not moved from either their home cages or environments.

Experiment Two

Twelve male rats of the Sprague-Dawley strain were divided randomly into two groups. Upon arrival in the laboratory, all rats were given distilled, deionized water and powdered Purina Laboratory Chow (#5001) ad libitum. One week was allowed for the animals to adjust to their new environment. All rats were housed individually. After one week, all animals were placed on a special Hartroft sodium test diet (United States Biochemical Corp., Cleveland, OH) containing 0.3% sodium, i.e. the same concentration as that in Purina Laboratory Chow.

Systolic blood pressures were measured from unanesthetized rats indirectly from the tail as described previously (Chapter Two). Pressures were recorded twice each week and represent the mean of ten measurements per animal. At the end of the measurements, the animals were immediately returned to their home environments.

Body weight, food and water intakes, as well as urinary volume were determined as described previously. Measurements were made daily at 0800 h except for the days on which blood pressure was measured.

After baseline measurements were made (one week), animals were moved into temperature controlled environmental chambers as described previously. At this time, the cold-treated group was placed on a reduced sodium Hartroft diet containing 0.15% sodium. Since cold-treated rats eat approximately twice as much food as controls, the intake of sodium would approximately be the same. Controls remained on the Hartroft diet containing 0.3% sodium. Measurements were continued for 21 days.

At the end of each experiment, animals were sacrificed and the organs rapidly removed and handled as described previously. Blood was collected from the trunk into chilled tubes containing either tetrasodium EDTA or no anticoagulant. The samples of blood were quickly placed into a chilled centrifuge and spun at 26,000 rpm for 45 minutes. The plasma and serum were transferred into labeled polypropylene tubes and stored at -80°C for later determination of aldosterone, osmolality and concentration of sodium and potassium in serum. In addition, two micro-hematocrit tubes were filled with blood collected from the trunk for the determination of hematocrit.

The concentration of aldosterone in plasma was measured in unextracted samples by radioimmunoassay. Samples (250 ul) were assayed in duplicate where possible. The gamma-coat kit (Diagnostic Products Co., Los Angeles, CA) has a detection limit of 25 pg/ml. Any value which measured less than 25 pg/ml was recorded as 25 pg/ml. Coefficient of variability for intraassay measurements was 3-8% and interassay variability was 4-10%. Osmolality was determined by a vapor pressure osmometer (Model 5100C, Wescor Co., Logan UT). Duplicate samples (5 ul) of serum were used. The osmometer has a precision of ± 2 mmol/Kg.

Statistical analyses of systolic blood pressure, body weight, and urinary outputs of sodium and potassium were carried out by a two-way analysis of variance repeated in time. Changes in organ weights, osmolalities, aldosterone concentrations of sodium and potassium in serum as well as hematocrit, were determined by a one-tailed Student's t-test for independent samples, when appropriate. Significance was set at the 95% confidence level.

Results

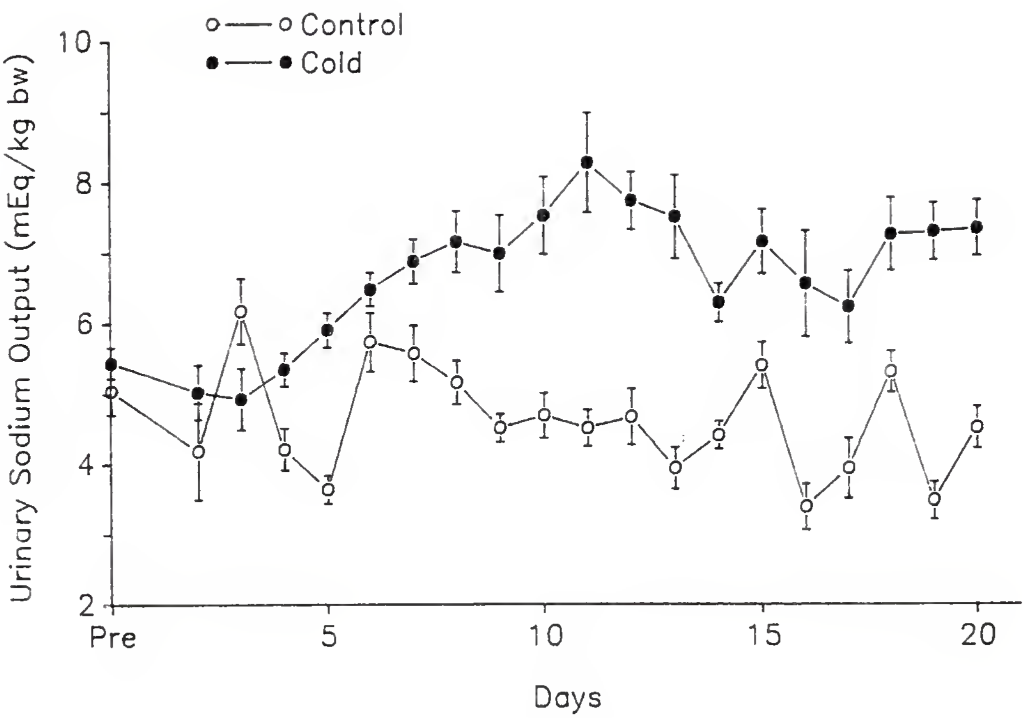
Experiment One

Chronic exposure to cold resulted in an increased daily consumption of food by an average of 50% (Table 3-1). Thus, the intake of sodium was 50% greater in the cold-exposed group. This increased intake was not associated with an increase in fluid intake of the same magnitude. Cold-exposed rats increased their average intake of water by only 20%. In addition, the average daily output of urine increased by greater than 50%. Associated with an increased intake of food, the 24 hour urinary output of sodium and potassium were significantly increased, as demonstrated by a significant ($p<0.01$) cold X time interaction and main effect (Figure 3-1, 3-2). The peak output occurred at the eleventh day of exposure, declined slightly but remained significantly greater than

Table 3-1. Effect of exposure to cold on daily food and water intakes and the outputs of urinary sodium and potassium.

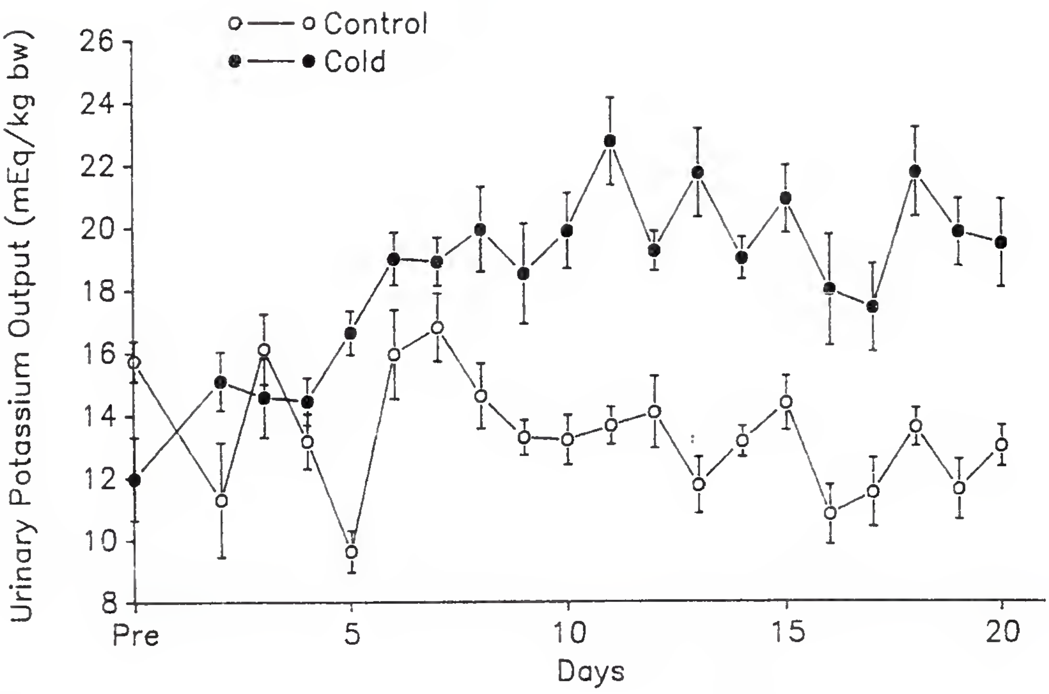
	Body Weight (g)	Food (g/100 g)	Water (ml/100 g BW)	Urine Volume (ml/100 g BW)	Urinary Sodium (mEq/Kg/day)	Urinary Potassium (mEq/Kg/day)
Purina Laboratory Chow						
COLD	376 ± 10	10.0 ± 0.2	14.7 ± 0.6	7.7 ± 0.5	6.7 ± 0.4	18.5 ± 1.1
CONTROL	396 ± 6	5.9 ± 0.1	10.4 ± 0.6	4.1 ± 0.4	4.6 ± 0.3	13.4 ± 0.9
Hartroft Diet						
COLD	406 ± 7	7.2 ± 0.3	6.3 ± 0.5	4.4 ± 0.4	nd	nd
CONTROL	426 ± 14	4.7 ± 0.2	7.4 ± 0.8	2.7 ± 0.3	nd	nd

Values are Mean ± S.E.M. and represent a three-day average.
nd = not determined.



Source	SS	DF	MS	F	P
BETWEEN					
Environment	396.33	1	396.33	51.77	<0.01
Error	130.16	17	7.66		
WITHIN					
Time	123.78	19	6.52	5.13	<0.01
Env.*Time	144.81	19	7.62	6.00	<0.01
Error	410.06	323	1.27		

Figure 3-1. The effect of 21 days of exposure to cold on the daily output of sodium in the urine of rats.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN Environment	2460.9	1	2460.9	62.9	<0.01
Error	665.13	17	39.13		
WITHIN Time	941.85	19	49.57	5.09	<0.01
Env.*Time	1076.66	19	56.67	5.82	<0.01
Error	3145.31	323	9.74		

Figure 3-2. The effect of 21 days of exposure to cold on the daily output of potassium in the urine of rats.
Values are Mean ± S.E.M.

controls throughout the duration of the experiment. This peak occurred later in time than did the peak for food and water intake which occurred by the fifth day of exposure to cold. A regression analysis of intake of sodium in food versus output of sodium in urine revealed no change in this relationship for the two groups (Figure 3-3). The cold-treated animals consumed more sodium and similarly had a greater output of sodium in the urine.

Daily refractive indices were measured throughout the period of exposure to cold to assess the concentration of urine. No significant main effect of cold air, nor a cold X time interaction was seen (Figure 3-4).

Experiment Two

The effect of administration of a reduced sodium diet during chronic exposure to cold on blood pressure is shown in Figure 3-5. Systolic blood pressure of the cold-treated group increased significantly ($p<0.01$) as indicated by both a significant cold X time interaction and main effect. All animals in the cold-treated group were hypertensive by day 21, as demonstrated previously.

The cold-exposed group significantly increased (54% over controls) their intake of food (Figure 3-6). In contrast to the earlier study using Purina Laboratory Chow (#5001), the cold-treated group did not significantly increase its intake of water (Figure 3-7). However, the increased urine output demonstrated in the earlier studies by the cold-exposed group was seen here as well (Figure 3-8). The rate of gain of body weight in the cold-treated group was significantly less than that of controls, as indicated by a significant cold X time interaction (Figure 3-9).

The effect of chronic exposure to cold on organ weights was not influenced by the reduced sodium diet. As seen previously, the ratios of organ weight to body weight for the kidneys, heart, right and left ventricles, atria and adrenal glands were significantly increased (Table 3-2). No difference in the weight of the thyroid gland was detected. Due to differences in body weights, regression analyses of each organ weight (mg) on body weight (g) were performed. No differences in slopes were detected for any of the

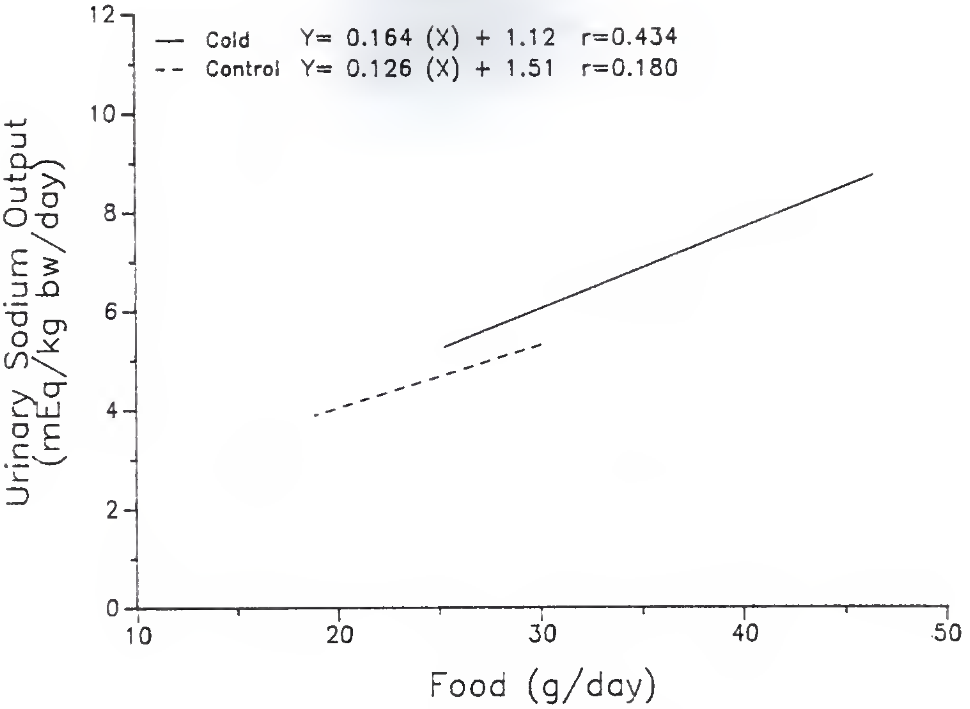


Figure 3-3. The effect of 21 days of exposure to cold on the relationship between the daily intake and output of sodium as determined by linear regression analysis.
Values are Mean ± S.E.M.

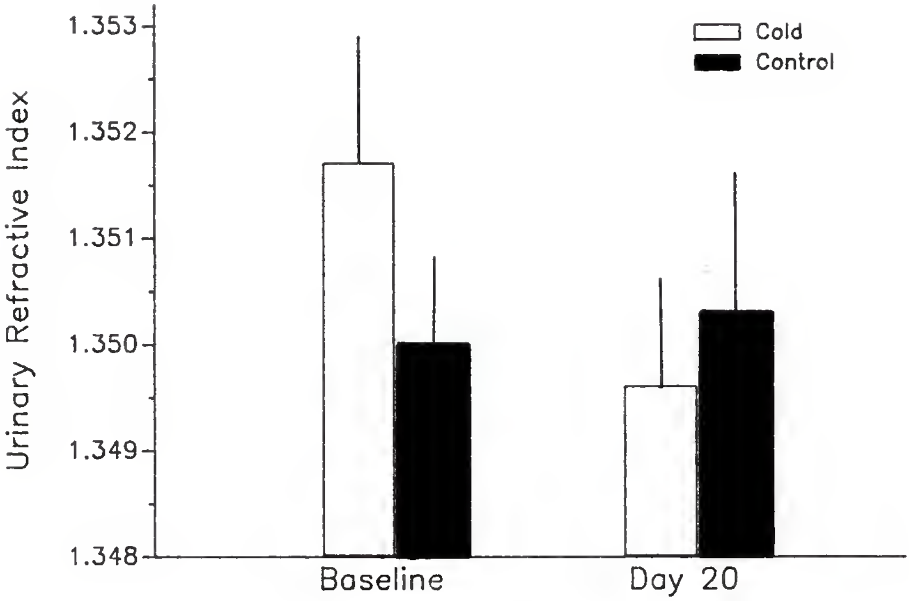
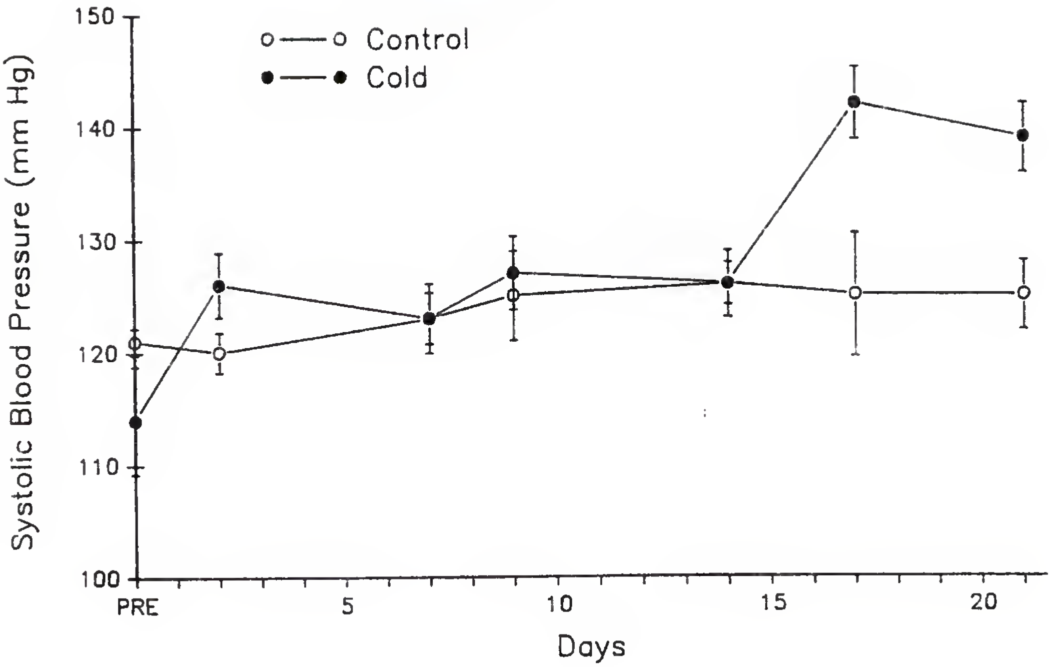
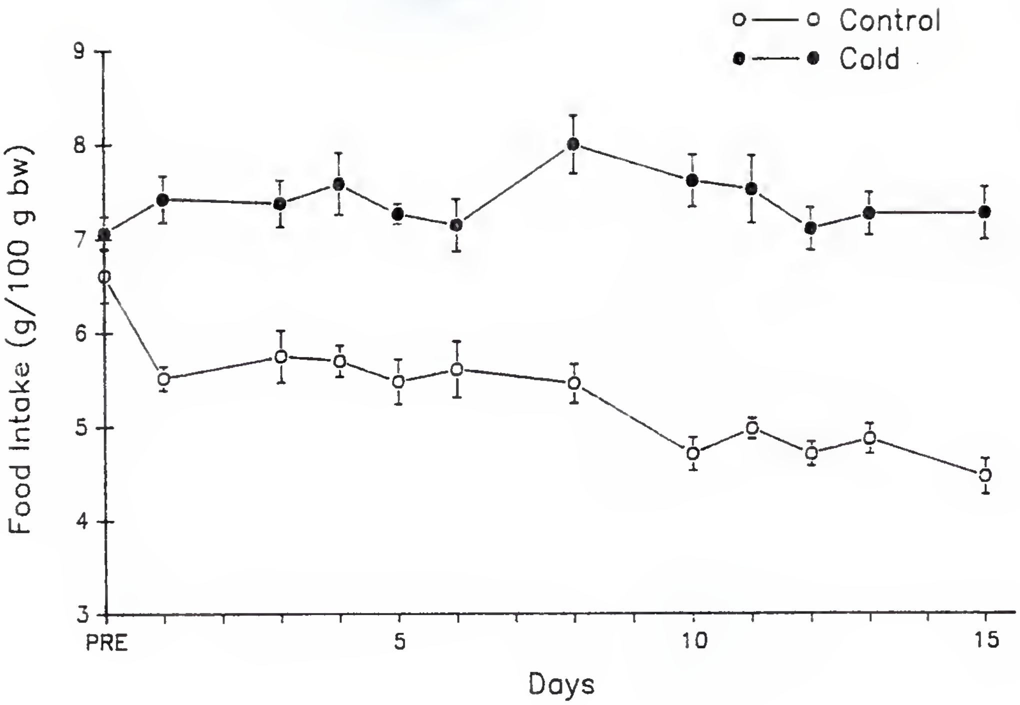


Figure 3-4. The effect of 20 days of exposure to cold on the refractive index of the urine of rats.
Values are Mean ± S.E.M.



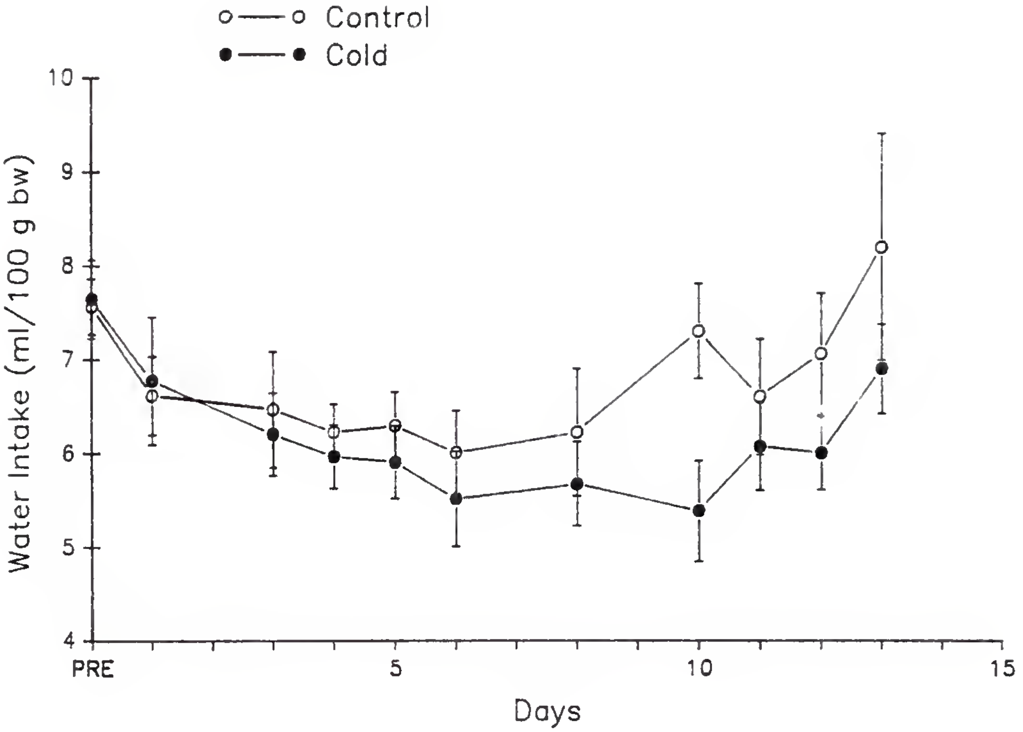
Source	SS	DF	MS	F	P
BETWEEN					
Environment	901.78	1	901.78	13.17	<0.01
Error	616.1	9	68.45		
WITHIN					
Time	2240.98	7	320.14	5.58	<0.01
Env.*Time	1479.5	7	211.35	3.684	<0.01
Error	3614.3	63	57.37	3.68	<0.01

Figure 3-5. The effect of 21 days of reduced sodium diet and exposure to cold on systolic blood pressure of rats. Values are Mean ± S.E.M.



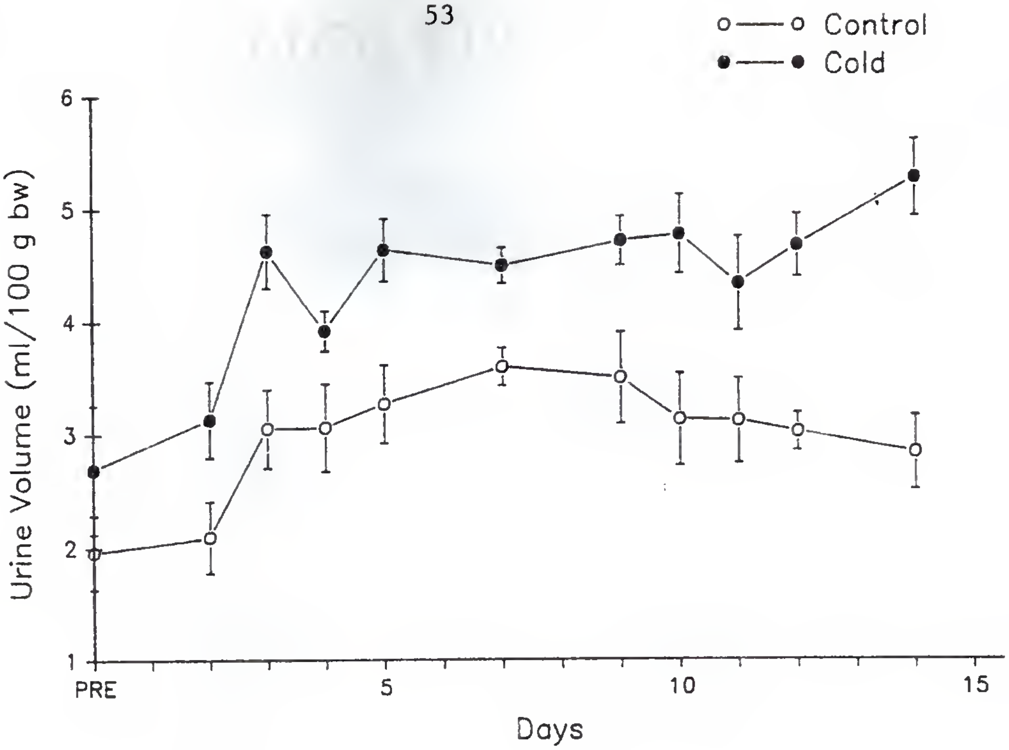
Source	SS	DF	MS	F	P
BETWEEN					
Environment	108.86	1	108.86	52.31	<0.01
Error	18.73	9	2.081		
WITHIN					
Time	9.141	11	0.831	4.95	<0.01
Env. * Time	23.845	11	2.168	12.91	<0.01
Error	16.588	99	166.2		

Figure 3-6. The effect of a low sodium diet during exposure to cold on the daily intake of food of rats.
Values are Mean ± S.E.M.



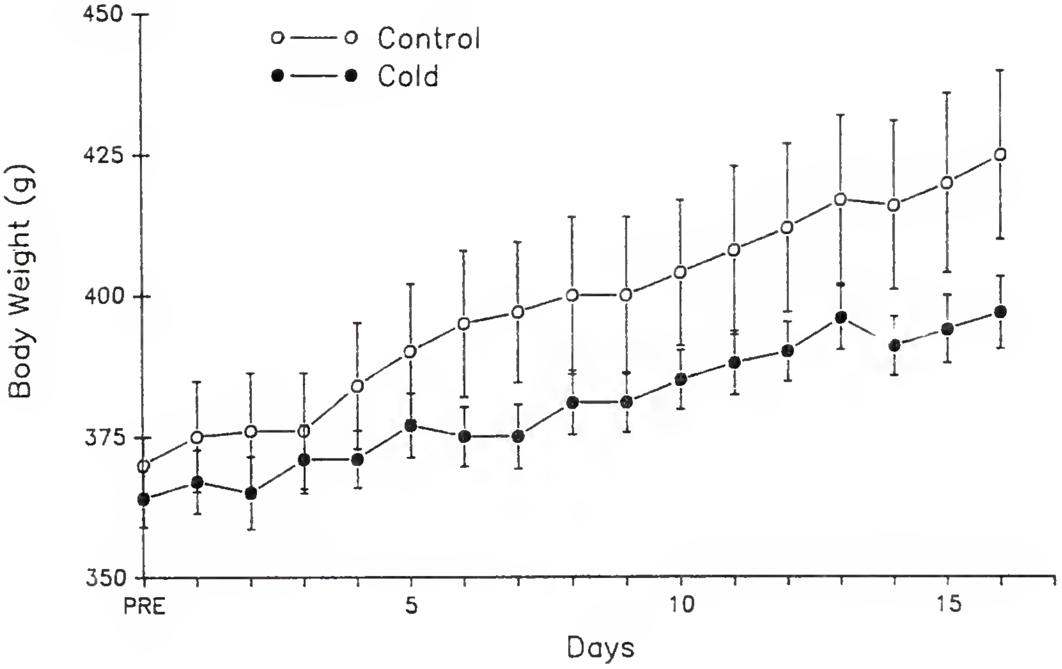
Source	SS	DF	MS	F	P
BETWEEN					
Environment	12.070	1	12.070	0.966	
Error	87.420	7	12.489		
WITHIN					
Time	34.714	11	3.156	4.732	<0.01
Env. * Time	15.544	11	1.413	2.118	<0.05
Error	51.370	77	0.667		

Figure 3-7. The effect of a low sodium diet during exposure to cold on the daily intake of water of rats.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN					
Environment	11.239	1	11.239	1.329	
Error	59.197	7	8.457		
WITHIN					
Time	40.920	11	3.720	11.735	<0.01
Env. * Time	5.016	11	0.456	1.438	
Error	24.377	77	0.317		

Figure 3-8. The effect of a low sodium diet during exposure to cold on the daily output of urine of rats.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
BETWEEN					
Environment	0.967	1	0.967	1.488	
Error	5.847	9	0.65		
WITHIN					
Time	7.052	15	0.47	117.50	<0.01
Env. * Time	0.285	15	0.019	4.750	<0.01
Error	0.488	135	0.004		

Figure 3-9. The effect of a low sodium diet during exposure to cold on the body weight of rats.
Values are Mean ± S.E.M.

Table 3-2. The effect of 22 days of exposure to cold on various organ weights of rats.

	Heart	Left Ventricle	Right Ventricle	Atria	Adrenals	Kidneys	Thyroid
			organ weight (mg)/(100 g) body weight				
COLD	348.9±8.9*	202.0±9.3*	45.0±2.9*	18.1±1.4*	18.6±0.9*	946.1±40.4*	5.2±0.2
CONTROL	297.8±6.9	174.4±5.6	35.0±1.5	11.2±0.6	13.4±1.6	771.9±27.4	4.6±0.3

Values are Mean ± S.E.M.
* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.

relationships, while the intercept was significantly greater in the cold-treated group for the whole heart (Table 3-3, Figure 3-10). When a regression analysis was carried out on final systolic blood pressure (X) versus heart weight (Y), no change in the relationship was found in the group chronically exposed to cold air. Simply, the cold-treated animals moved to a higher position along this relationship. However, when a regression analysis was performed on final systolic blood pressure (SBP) and the weight of the left ventricle, a significant effect ($p<0.05$) of exposure to cold on the intercept was evident. Thus, at any SBP, the weight of the left ventricle of the cold-treated group was greater (Figure 3-11).

There was no significant effect of the reduced sodium diet either on the concentrations of sodium and potassium in serum or on serum osmolality (Table 3-4). In addition, no difference in hematocrit was seen in cold-treated rats in this study (Table 3-4). This finding is different from that seen earlier when cold-exposed rats were given Purina Laboratory Chow (0.3% sodium). The concentration of aldosterone in the plasma was not affected by exposure to cold in either experiment. No significant effect of a low sodium intake was seen on the level of aldosterone. Although the trend was toward an increased concentration, the variability was quite large.

Discussion

The association between the intake of dietary sodium and the prevalence of hypertension and cardiovascular disease has been suggested by epidemiological and clinical studies. The use of sodium restriction and diuretic administration as effective antihypertensive therapies in humans also supports the idea of a role for sodium in hypertension. It also suggests a role for volume reduction. In addition, several experimental models are dependent on an increase in sodium intake including the Dahl salt-sensitive and DOCA/salt models. When animals are chronically exposed to low environmental temperature there is an immediate and sustained increase in metabolic rate. The increased metabolic rate, and thus caloric expenditure, necessitates an increase in the intake of calories via food. The laboratory rat is restricted in its choice of food in that

Table 3-3. The effect of 22 days of exposure to cold on the relationship between organ weight and body weight.

Organ Weight (mg) = B(1) x Body Weight (g) + B(0)

	B(1)		B(0)		Correlation Coefficient	
	Control	Cold	Control	Cold	Control	Cold
Whole Heart	2.84	3.17	68.14	126.71*	0.912	0.546
Left Ventricle	1.79	3.15	-12.90	-460.00	0.845	0.534
Right Ventricle	0.28	0.31	30.31	52.11	0.605	0.189
Atria	0.10	0.33	7.51	-60.92	0.550	0.400
Kidneys	13.88	27.27	-2565.00	-7232.00	0.966	0.865
Adrenal Glands	0.46	0.52	-135.00	-136.70	0.866	0.789

* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.

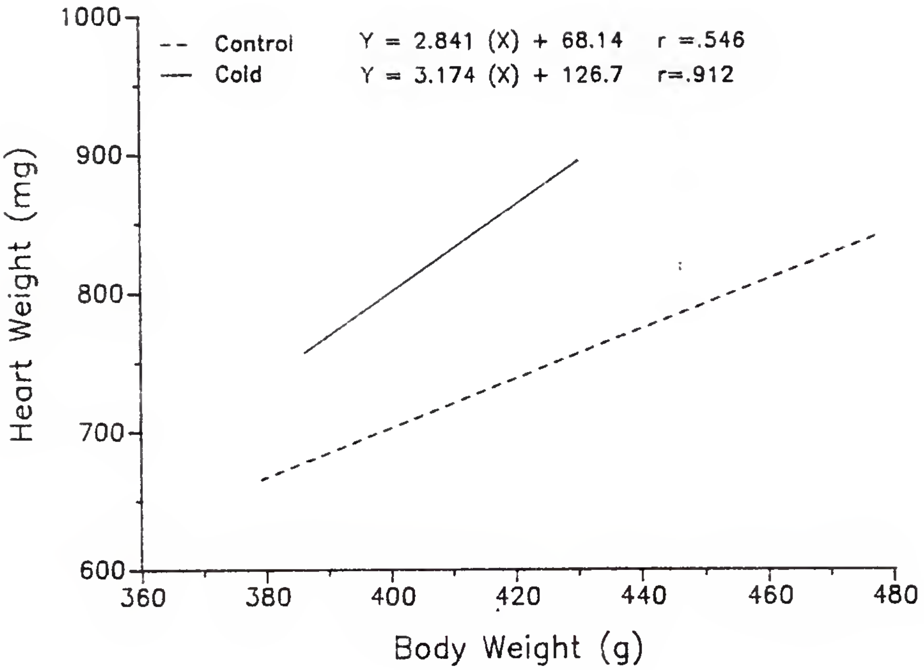


Figure 3-10. The effect of chronic exposure to cold on the relationship between body weight and the weight of the heart as determined by linear regression analysis.

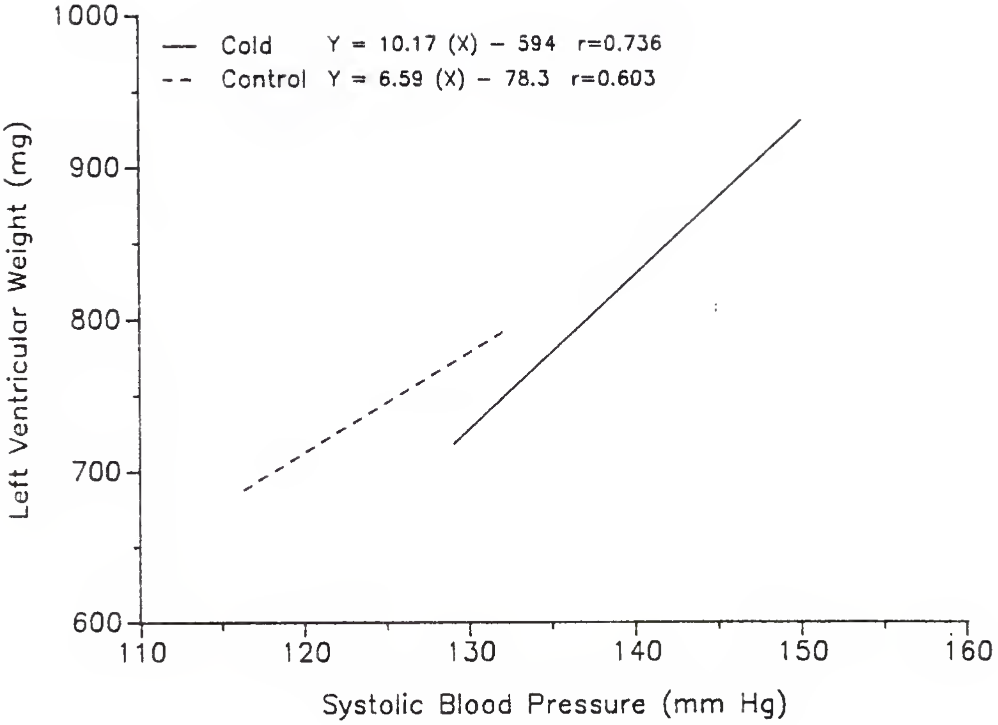


Figure 3-11. The effect of chronic exposure to cold on the relationship between systolic blood pressure and the weight of the left ventricle as determined by linear regression analysis.

Table 3-4. Effect of chronic exposure to cold and a reduced sodium diet on hematocrit, serum electrolytes, osmolality and plasma aldosterone concentration.

	HEMATOCRIT	SODIUM mEq/L	POTASSIUM mEq/L	OSMOLALITY mOsm/Kg	ALDOSTERONE pg/ml
Purina Laboratory Chow					
COLD	48.8 ± 0.9*	138.5 ± 0.6		283.9 ± 1.9	71 ± 16
CONTROL	46.8 ± 0.4	140.1 ± 0.3		284.1 ± 1.3	45 ± 9
Hartroft Diet					
COLD	45.9 ± 1.4	137.0 ± 0.7	8.4 ± 0.7	284.2 ± 1.3	192 ± 95
CONTROL	45.2 ± 0.2	139.2 ± 1.0	7.5 ± 0.4	285.0 ± 1.4	39 ± 13

Values are Mean ± S.E.M.
* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.

the chow provided is its sole source. Thus the composition of the diet is also restricted in the content of sodium. The increased intake of food is accompanied by an increased intake of sodium. In the first series of experiments, food and thus sodium intake increased by over 50%. It was possible that changes in sodium intake of such magnitude contributed to the elevation of blood pressure seen in this model. Hence, a low sodium diet was employed to investigate this possibility.

In the first experiment, both groups (control and cold) were given identical Purina Laboratory Chow (#5001) with a sodium content of 0.3%. The cold-treated group consumed over twice as much sodium as controls. In addition, the cold-treated group consumed only 20% more water while excreting 50% more urine. The shift in the relationship between food:water intake and water intake:urine output supports the idea of self-imposed volume contraction as originally proposed by Fregly et al. (58). It would be difficult for the cold-treated animal to be volume contracted while consuming a large amount of sodium, the primary regulator of extracellular fluid status, unless the excess sodium could be eliminated. When urinary sodium and potassium outputs were measured, the cold-treated group excreted 45% and 40% more sodium and potassium, respectively. In addition, when a linear regression analysis compared the output of urinary sodium (Y) against the intake of sodium or food (X), no difference in the relationship was evident in the cold-treated animals (Figure 3-11). Thus, the increased consumption of sodium was effectively offset by an increased excretion. It does not appear as if the cold-treated animal retains the excess sodium it ingested. This would suggest that sodium retention is not a mechanism which contributes to cold-induced hypertension. The results do, however, suggest that extracellular fluid volume may be reduced in cold-treated rats.

Previous work with chronic exposure to cold suggests that a self-imposed imbalance in fluid exchange occurs (58). In the present studies, an increase in food and water intakes as well as an increase in urine output were demonstrated. One mechanism which would prevent dehydration in this situation would be to increase the output of solutes in the urine in a reduced urine output (i.e. increased urine osmolality). When the refractive index of the urine was measured, no difference in the urine concentration between groups was found. Similarly, Fregly et al. (58) found no difference in urine osmolality in cold-treated rats. Thus, the increased osmolar load delivered to the kidney as a result of the increase in food consumption was handled by an increase in urine volume not by excreting a more concentrated urine. This cold-induced dehydration has been suggested to occur in man (179,123,146,2) as well as in rats by other investigators and has been reviewed extensively (57).

A retention of sodium, and thus an increase in fluid volume, is only one mechanism by which sodium may contribute to hypertension. It is unclear whether the elevated intake of sodium must be retained in order to be a contributing factor in an elevation of blood pressure. Perhaps as a result of handling the increased sodium, changes have occurred at the cellular level or at the kidney. To investigate this possibility, a diet low in sodium (50% less than control) was administered to the experimental group during exposure to cold in experiment two. The cold-treated group consumed 54% more food than did controls. Thus, no difference in sodium intake occurred. Interestingly, no increase in the intake of fluid was demonstrated in this experiment suggesting that the increased water intake seen in the previous experiments is associated with the increased sodium intake. Urine output however, was significantly increased as seen with normal chow. These results further support a state of self-imposed dehydration.

The systolic blood pressure of the cold-exposed group was significantly increased in the presence of a normalized sodium intake. The blood pressure was increased by the seventeenth day of exposure to cold and remained elevated when the experiment was ended. The time-course, or the amount of exposure necessary for an elevation of blood pressure, was unchanged, as was the magnitude. In addition, significant hypertrophy of the whole heart, right and left ventricles, atria and adrenal glands was evident. When regression analyses were performed on the organ weight to body weight relationships, only the intercept for the whole heart was significantly increased. However, exposure to cold significantly altered the relationship between systolic blood pressure and left ventricular weight. When final systolic blood pressure (Y) was compared against left ventricular weight (X), the intercept was increased. This suggests a work-induced hypertrophy.

No effect of a reduced sodium diet during exposure to cold on either plasma electrolytes or the primary sodium regulating hormone, aldosterone, was found. Although the serum sodium concentration of the cold-treated group on a reduced sodium diet tended to be lower, it was not significantly so. Likewise, there was a trend for the aldosterone concentration of this group to be higher, but there was a large variability.

Recent studies investigating human essential hypertension have provided inconclusive evidence for a role of sodium. From such studies, two observations seem clear. First, not all individuals respond to an increase in sodium intake with an elevation of blood pressure. Second, sodium restriction and diuretic therapy are not effective in all hypertensive patients. In addition, not all experimental models of hypertension are dependent on an increased sodium intake. The present studies failed to indicate that an elevated sodium intake was associated with an elevation of systolic blood pressure. However, the present studies do not eliminate the possibility of an indirect effect of sodium in either this or other models of hypertension as intracellular sodium content, sodium transport changes, and other sodium dependent processes were not investigated.

CHAPTER FOUR
THE ROLE OF SYMPATHETIC NERVOUS SYSTEM IN COLD-INDUCED HYPERTENSION

Introduction.

It is well known that the sympathetic nervous system (SNS) controls both minute-to-minute and long term arterial pressure. In addition, there is a great deal of evidence which suggests that changes occur within the SNS in nearly every form of experimental hypertension (202). Much of the difficulty in assessing the exact role for the central nervous system in hypertension has been attributed to the inability to identify an area or region of neurons responsible for maintaining the normal output of the preganglionic sympathetic fibers and thus normal arterial pressure. The output of the SNS is controlled by a negative feedback loop with input from volume or stretch sensitive mechanoreceptors via the baroreceptor reflex. These receptors respond to local distention with an increased activity which is transmitted, via the IX and X cranial nerves, to the central nervous system. The first synapse of the baroreceptor system is in the nucleus tractus solitarius (NTS) located in the ponto-medullary region of the brainstem. An increased afferent activity in this region results in the stimulation of an inhibitory pathway (1,164). This increased inhibitory input is targeted toward the tonic activity of the sympathetic fibers which influence vasomotor tone and cardiac rate. It is well known that the NTS receives input from higher brain centers, including the area postrema, hypothalamus, thalamus, stria terminalis and cerebral cortex (1). The large amount of input to the region of the NTS led Abrams (1) to suggest it as the center which integrates central and peripheral information to change the cardiovascular system, and thus to maintain circulatory homeostasis. In addition, the NTS projects to a large variety of centers including the dorsal motor nucleus, parabrachial nucleus, hypothalamus, ventrolateral pressor area of the medulla, as well as direct projection to the spinal cord. Several of these areas are also involved in the control of body temperature as mentioned previously (Chapter Two). Thus, the potential for direct neural communication between the centers which are involved in temperature regulation and those involved in the regulation of blood pressure is an interesting possibility.

Over the last several years, Reis and his associates (164) have been identifying the regions, pathways, and neurotransmitters which are ultimately involved in controlling resting arterial pressure. Their work suggests that a specific zone within the rostral ventrolateral quadrant of the medulla oblongata functions in this capacity. Further, a subpopulation of adrenergic C1 fibers (which stain specifically for epinephrine (E)) directly innervate preganglionic neurons in the spinal cord (164). In addition, it is in this area, where clonidine, a clinically efficacious antihypertensive agent, acts to lower blood pressure (16,70,163). Interestingly, clonidine, a mixed alpha$_1$- alpha$_2$-adrenoceptor agonist works as an imidazole and thus at an imidazole receptor to lower arterial pressure rather than at an adrenergic receptor as originally hypothesized (190,46). With these new findings, it may now be possible to examine whether changes occur centrally which contribute to the elevated blood pressure in hypertensive animals. Further, these findings provide impetus for a renewed interest in the role of the baroreceptors in hypertension.

Several lines of evidence suggest a role for the baroreceptor reflex in hypertension. The first line of evidence stems from the basic idea that the baroreceptor reflex functions via a negative feedback mechanism to decrease the output of the SNS. Many forms of established experimental hypertension are characterized by a hyperactive SNS. A decreased inhibitory influence is one mechanism by which the baroreceptors could contribute to such a hyperactive state. Second, an attenuated baroreceptor reflex function has been demonstrated in many experimental models of hypertension. Whether the baroreceptor reflex is altered in cold-induced hypertension is unknown.

The role of the SNS is not simply a one mechanism hypothesis in which excessive activation or output acts on the vasculature directly to result in vasoconstriction.

Indeed, as many additional possibilities exist as there are functions for the sympathetic nervous system. For instance, an increased vascular reactivity has been reported in hypertensive humans (39) as well as in various models of experimental hypertension (60,110,49). The changes in vascular responsiveness may be due to changes in a) receptor levels; b) post-receptor transduction; c) direct neuromodulation (by AII, purines, prostaglandins or local metabolites); d) local temperature (cold facilitates vasoconstriction) and e) false transmitters. The exact mechanisms for the changes in vascular responsiveness in the various models and significance of those findings remains for further investigation.

It is also well known that the kidneys are highly innervated by the SNS. The effects of the adrenergic system on renal function, and thus arterial pressure during the development of hypertension is still under investigation. An increase in renal nerve activity may increase renal resistance, reduce glomerular filtration rate, increase plasma renin activity and directly mediate tubular sodium reabsorption. Additionally, acute occlusion of the renal artery (109), intravenous infusion of E (107), and electrical stimulation of the renal nerves (42) result in a decrease in sodium excretion. When NE was chronically infused into the renal artery, Cowley and Lohmeier (33) demonstrated a shift of the body fluid volume-arterial pressure function curve to the right and an elevation of blood pressure. A shift in the renal function curve indicates that an increase in arterial pressure would be necessary for any given level of sodium and water excretion during chronic infusion of NE in order to achieve long-term fluid and electrolyte balance. According to Guyton (75), the kidney (i.e. the renal function curve) is the ultimate regulator of arterial pressure. No sustained change in arterial pressure is possible unless a change has also occurred at the level of the kidney (78). Whether such changes occur in cold-induced hypertension remain to be investigated. However, if the curve is shifted to the right, this would provide a mechanism to explain how an elevated arterial pressure could be sustained in the face of the volume contraction as hypothesized in the cold-induced model (Chapters Two and Three).

Chronic exposure of rats to cold results in an elevation of blood pressure. In addition, these animals are characterized, at least in the acute phase of adaptation, as having a large increase in the activity of the SNS (134). Further, an elevated activity of the SNS, and thus, an increased circulating level of NE would imply an elevation of plasma renin activity and AII, as well as a decreased sensitivity of the baroreceptor reflex. Whether these alterations occur and are sustained, and whether they contribute to the elevation of blood pressure seen with exposure to cold, is unknown. The purpose of these experiments was to describe the blood pressure, baroreceptor and SNS profile of the cannulated rat which is chronically exposed to low environmental temperature.

Methods

General Methods

Twenty male Sprague Dawley rats weighing between 150-200 g were housed individually and provided with powdered Purina Laboratory Chow (#5001) and water ad libitum. The vivarium was maintained as described previously (Chapter Two). After the femoral arteries were cannulated, the rats were divided randomly into two groups (control or cold-treated). Ten days were allowed for them to recover fully from the effects of surgery. On the first experimental day, both groups were moved into temperature controlled environmental chambers (either 26 or 6°C).

Cannulation Procedure

Cannulae. Venous catheters were prepared from 50 cm lengths of 20 gauge clear vinyl tubing (SV-55, 0.8 mm ID, 1.2 mm OD, Dural Plastics, Australia). A slight bevel with a blunt tip was cut on one end. Arterial catheters were prepared from 3.5 cm lengths of vinyl tubing (SV-31, Dural Plastics) and connected to a long (48 cm) length of Tygon microbore tubing (S-54HL, 0.04" ID, 0.07" OD) with cyclohexanone. A small piece of thin wall, stainless steel hypodermic tubing (HTX-24TW, Small Parts INC, Miami, FL) was inserted at the SV31/SV55 junction, this allowed the suture to be secured tightly without collapsing the cannula. The cannulae were gas-sterilized (ethylene oxide) prior to use.

Cannula sheaths. The catheters were pulled through a sterilized (ethylene oxide) steel spring coil (12 in. length, 0.26 in. OD, 0.206 in. ID, Exacto Spring Co, Grafton, WI) with a dacron Patch (0.8 mm thick, Troy Mills, NH) attached to one end. The dacron patch was implanted subcutaneously; sutured at two points to secure the catheter/spring in place, and the skin sutured over it. The spring and catheter were exteriorized at the front of the cage. The spring coils were lightly coated with quinidine sulfate (200 mg/ml ethanol) to discourage chewing.

Surgery and maintenance of cannulae. Animals were fasted the night before surgery. All surgery was conducted in a semi-sterile designated surgical area. Surgical instruments were sterilized either in activated dialdehyde solution (Cidex, Surgikos, Arlington, TX) for 24 hours or autoclaved prior to use. Animals were initially anesthetized with Brevitol HCl (50 mg/kg, Lilly) given i.p. and maintained with Sodium Pentothal (0.8 mg/kg, Abbott Laboratories, N. Chicago, IL) i.v. as needed. Once anesthetized, a small area of the left inner leg and an area between the shoulders was shaved and then cleansed with Zephiran Chloride solution (Winthrop Labs, New York, NY). Sterile catheters were implanted first into the femoral vein and then into the femoral artery. The femoral vein was separated from the femoral nerve and artery by blunt dissection; preservation of nearby nervous tissue was emphasized. The cannula (filled with sterile saline) was inserted approximately 35 mm into the femoral vein. Local application of Lidocaine HCl (0.2%, Dexter Corp, Chagrin Falls, OH) was applied to the isolated vessel to prevent vascular contraction. Once the catheter was inserted, it was secured with sterile 5-0, monofilament nylon suture (Ethilon 698G, Ethicon, NJ). A small portion of the femoral artery was dissected from the femoral nerve. The catheter was inserted into the artery and secured proximally and distally with sterile 5-0 suture. The free end of the catheters were brought around to the back subcutaneously via a trocar and exteriorized through a small incision between the shoulder blades. Upon completion of surgery, animals received 15 mg of Ampicillin (50 mg/ml, Bristol Labs, NY) given subcutaneously. Bacitracin/Polymyxin B/Neomycin (ointment, Upjohn Co., Kalamazoo, MI) and 2% Xylocaine Jelly (lidocaine HCl, Astra Pharmaceutical Products INC, Westborough, MA) were applied to all skin incisions which were closed with wound clips (9 mm, Clay Adams, Parsippany, NJ). These precautions aid in prevention of infection, minimized the discomfort to the animals and permitted long-term sampling. Catheters were flushed with sterile heparin sodium (10 USP U/ml of 0.9% saline), filled with sterile heparin (1000 u/ml, porcine derived, LyphoMed INC, Rosemont, IL), and sealed with either an 18 or 20 gauge plug. All animals were closely monitored following surgery. Any animal which appeared to experience more than the normal discomfort associated with the surgical procedures was euthanized with an overdose of pentobarbital. The use of a chronic cannulated preparation permitted easy access to arterial blood samples, i.v. administration of drugs, and direct recording of blood pressure without disturbing the animal. In addition, it allowed maximal freedom of movement for the rat. Using a similar catheterization procedure and the carotid artery, this author has kept cannulae patent for at least 6 weeks. The described procedure is quite similar to that of Raff et al. (159,160) who have maintained cannulae patent in the femoral artery until their experiment ended (30 days). As regards attrition, over 80% of the arterial and venous cannulae were patent after 42 days in the controls, while only 50% of the cold-treated group had both cannulae functioning at this time. Although this is the first report of this difference between normo- and hypertensive animals with regard to functional life of cannulae, it has been mentioned by Weeks (unpublished, personal communication No. 1011, UpJohn) as well.

Sample Collection

Arterial blood samples (< 5 ml/kg) were collected at various times throughout the experiment. At 0800 h, the dead space of the arterial line (0.4 ml) was removed and discarded. An additional 0.4 ml of blood was withdrawn into a sterile 1 ml syringe and set aside. The arterial sample was withdrawn into a 3 ml syringe containing tetrasodium EDTA (50 ul 0.3 M/ml sample, Sigma, St. Louis, MO). Care was taken not to disturbed the animal. The samples were immediately placed into chilled polypropylene tubes. In addition, two micro-hematocrit tubes (ammonium heparin) were filled directly from the cannula. The 0.4 ml of blood which was removed earlier was returned to the animal. A volume of sterile saline equal to the sample volume taken was administered. The saline served to maintain volume and to flush the cannula. The cannula was refilled with heparin (1000 U/ml) and sealed. Blood samples were quickly placed into a chilled centrifuge (IEC Model CRU-5000) and spun at 26,000 rpm for 45 minutes. The plasma was removed and placed into labeled polypropylene tubes and stored at -80°C until analysis of plasma catecholamines by high pressure liquid chromatography with electrochemical detection. After the hematocrit tubes were spun and the data recorded, the plasma was removed and stored in micro-tubes for later determination of its sodium and potassium concentrations.

Direct Blood Pressure Recording

Throughout the experiment, arterial blood pressure was measured in the respective environment of the animal (26 or 6°C). At 0800 h, the contents of the arterial cannula were voided. The cannula was flushed with sterile saline and refilled with heparin/saline (10 U/ml). The cannula was connected to a physiological pressure transducer (Model RP1500, Narco-BioSystems, Houston, TX) with input into a low level DC preamp and a Grass Model 5D, 4 channel direct-writing recorder polygraph. Systolic, diastolic and mean blood pressures, as well as heart rates, from undisturbed animals which remained in their home cage were recorded. After the measurements were completed, the catheters were flushed with sterile saline and then refilled with heparin (1000 U/ml) and sealed.

Baroreflex Function

Baroreflex function was assessed at several times during the experiment by pharmacological manipulation of blood pressure using a procedure similar to Smyth et al. (178). Arterial cannulae was flushed with sterile saline and refilled with heparin/saline (10 U/ml). The cannula were connected to a 3-way stopcock and then to a Statham pressure transducer (P231D, Statham Instruments, Oxnard, CA). The transducer was connected to a Gould recorder. Baroreflex tests were recorded directly online to an IBM

PC computer via an analog-digital converter (Keithley model 507, Keithley DAS, Cleveland, OH). Sampling rate was 10 Hz. Blood pressure was increased by i.v. administration of phenylephrine (2 ug/kg, Winthrop-Breon Laboratories, New York, NY) diluted at a concentration so that the volume given was less than the dead space of the venous catheter. The phenylephrine was flushed with sterile saline into the animal. A baroreflex function-curve was constructed by plotting the heart period (1/heart rate) against mean arterial pressure at any given time. The slope and intercept of the relationship was determined by linear regression using a scientific software package (Assystant Plus, Version 1.04, Assystant Scientific Software Co.). The slope was used as an indication of the sensitivity of the gain of the reflex, and the intercept as an indication of setpoint (84).

Daily Intakes

Daily food and water consumption, urinary volume, creatinine and catecholamine excretions of individual rats were measured. Measurement of daily intakes were described previously (Chapter Two). Urine was collected in graduated cylinders which contained 0.25 ml of 3 N HCl. After total urine volume was recorded, 5 ml aliquots were frozen for later analysis of norepinephrine and epinephrine by high-pressure liquid chromatography with electrochemical detection (HPLC-EC).

Measurements

Plasma Catecholamines. Norepinephrine (NE) and epinephrine (E) concentrations in plasma were determined by HPLC-EC. The procedure used is a simple extraction with acid washed alumina (AAO) as originally suggested by Anton and Sayre (4) with later modifications (12,145). Basically, the sample and internal standard were placed in vials and adsorbed onto AAO directly from the plasma at pH 8.5. After shaking, the alumina is washed with water and the catecholamines eluted with a small volume (200 ul) of acid (0.1 N $HClO_4$, Fisher #A229). The acid extract contained the catecholamines and was injected directly into the HPLC. This procedure is selective for the parent amines and allows for preconcentration to a small volume. The procedure is described in LCEC Application Note No. 14 available from Bioanalytical Systems INC.; the entire procedure appears in Appendix A. Dihydroxybenzylamine (DHBA, Sigma) was added to each sample prior to extraction, served as the internal standard, and permitted adjustments in recovery. A previously collected plasma pool as well as standards were assayed with each run of 10 samples. This permitted the determination of intra- and interassay coefficient of variance (Appendix A).

Chromatography. A high pressure liquid chromatograph equipped with a LC-4 electrochemical detector (HPLC-EC) and a glassy carbon electrode (Bio Analytical Systems, Lafayette IN) was used in all determinations. The electrode potential was 0.7 volts run at a sensitivity of 2 nAmps. This potential allowed for optimal recovery. The solvent delivery system consisted of a Bio-Rad Model 1330 pump (Bio-Rad, Richmond, CA) connected to a Bio Sil reverse-phase ODS-10 column (250 mm x 4 mm, Bio-Rad #1250082). The mobile phase consisted of 0.1 M potassium dihydrogen phosphate (Fisher #P285) buffer:methanol (95:5) containing 0.1 mM EDTA (Sigma #ED4SS) and 5 mM heptane sulfonic acid (Fisher #O-3013). The pH was adjusted to 3.8 with 2 M phosphoric acid prior to the addition of methanol. The mobile phase was filtered prior to the addition of the ion-pairing agent and degassed prior to use. After an initial equilibration period, the mobile phase was recycled during use. The linearity of the detector, detection limits, recovery and intraassay coefficient of variance were established prior to assaying samples. The results appear in Appendix A. Results are expressed as ng/ml.

Urinary Catecholamines. The concentrations of catecholamines in 24 hour urines were determined by HPLC-EC from samples after the catecholamines were isolated and preconcentrated. Basically, the catecholamines were isolated on cation resin exchange columns (Biorex 70 cation resin, Bio-Rad) eluted with 2 M ammonium sulfate. The catecholamines were adsorbed on AAO as described above and the resultant acid extract injected into the HPLC. The internal standard DHBA was added to each sample prior to column extraction. Results are expressed as ng/day.

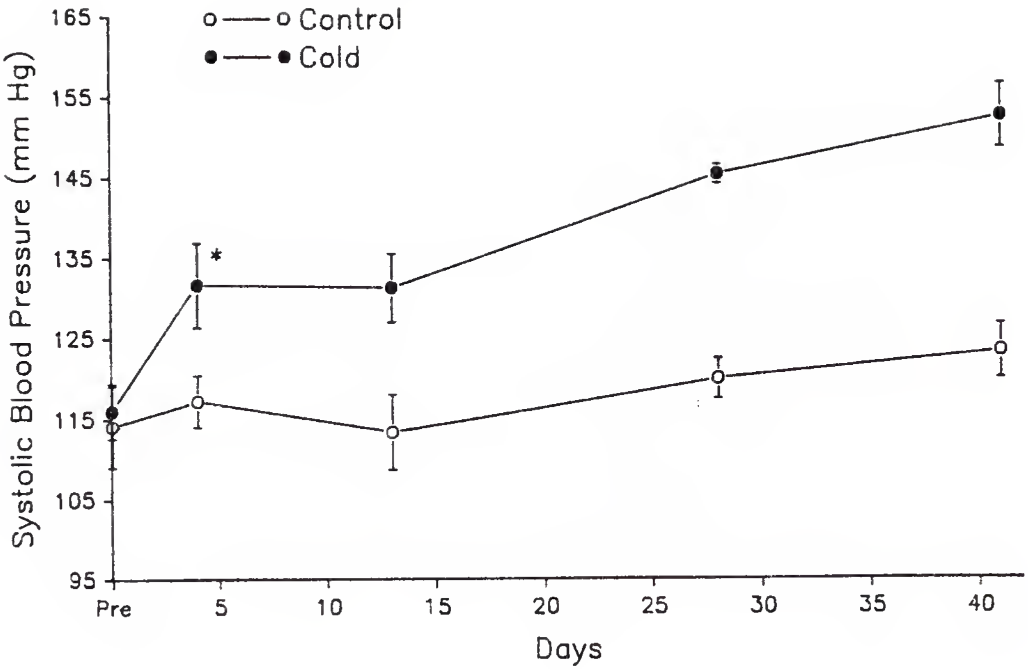
Creatinine. The concentration of creatinine in urine and plasma was determined by a method described by Technicon Corporation (No.SE4-0011FH4). The results were expressed as mg/kg body weight.

Statistics

Statistical analyses of data were carried out by a two-way randomized block design, analysis of variance which tested for the main effects of environment and time. A posteriori pairwise comparisons were performed when appropriate by a Duncan's New Multiple Range test which maintained the significance at the a priori level ($p < 0.05$) (111). Changes in the organ weights, and in the slopes and intercepts of regression analyses were determined by a one-tailed Student's t-test for independent samples, when appropriate. The hypothesis was that the variable being tested in cold-treated animals was less than or equal to that of controls (H_0: cold $\leq$ control). Significance was set at the 95% confidence level.

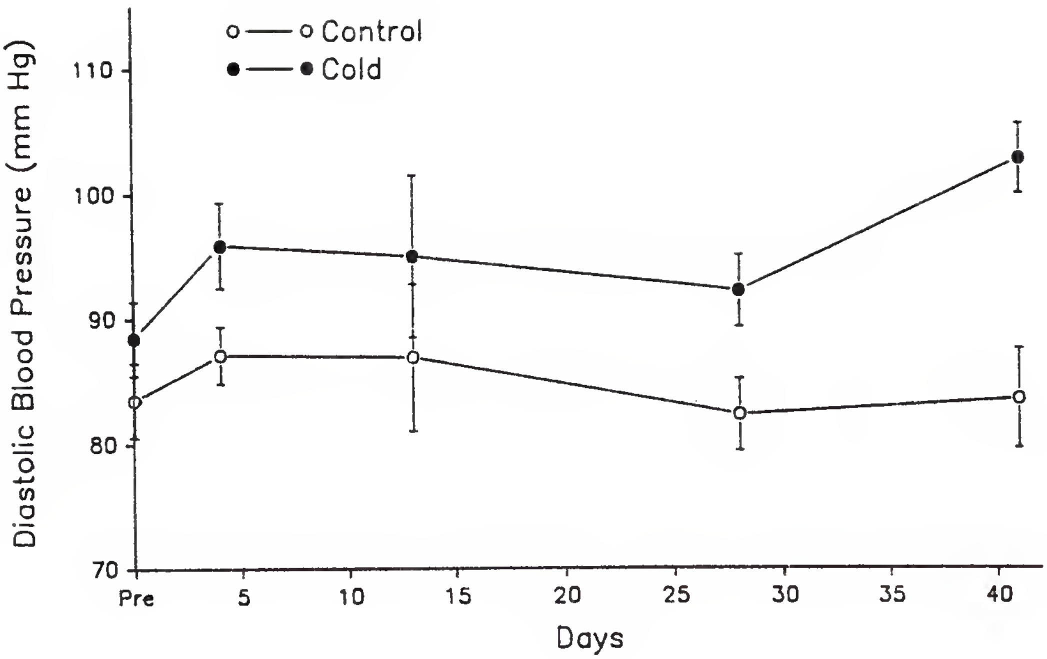
Results

The effect of exposure to cold on systolic and diastolic blood pressures is shown in Figures 4-1, 4-2. Systolic blood pressure was significantly increased above that of controls on day four, the earliest time a measurement was made. Systolic blood pressure remained elevated and relatively constant when measured again 10 days later. Systolic blood pressure then increased to 146 ± 1 mm Hg (day 28), and to 153 ± 4 mm Hg (day 41). Diastolic blood pressure increased rapidly from a resting level of 90 ± 3 mm Hg to 96 ± 3 mm Hg by day four and remained unchanged until day 41 when a large increase (103 ± 3 mm Hg) was demonstrated. The effect of exposure to cold on mean arterial pressure is shown in Figure 4-3. Mean arterial pressure was significantly increased by exposure to cold as indicated by a significant interaction and main effect. Mean arterial pressure increased from pre-cold levels of 101 ± 4 to 111 ± 3.5 mm Hg on day four and continued to increase up to 124 ± 2 mm Hg when the experiment ended. The positive slopes for systolic and mean arterial pressures suggest that even after 41 days of exposure to cold, the blood pressure had not reached its maximum.



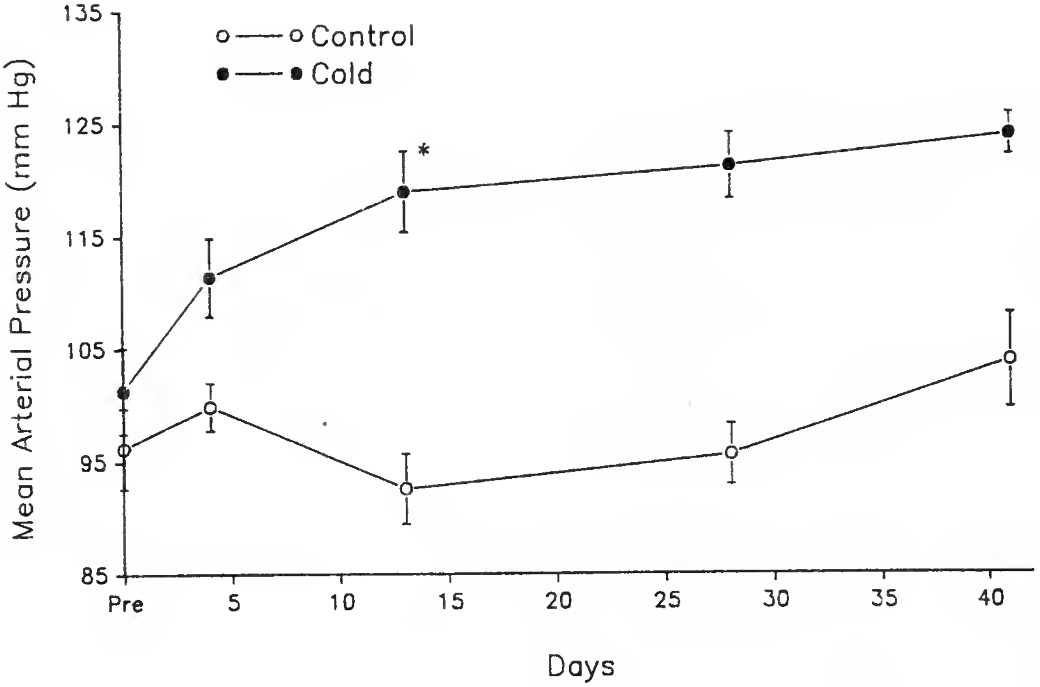
Source	SS	DF	MS	F	P
Environment	5509.9	1	5509.9	43.86	<0.01
Time	4411.9	4	1102.9	8.78	<0.01
Env. * Time	1553.28	4	388.32	3.09	<0.05
Error	8040.75	64	125.64		

Figure 4-1. The effect of chronic exposure to cold on systolic blood pressure of chronically cannulated rats.
Values are Mean ± S.E.M.
* First point which is significantly greater ($p < 0.05$) than control as determined by Duncan's New Multiple Range test.



Source	SS	DF	MS	F	P
Environment	1731.8	1	1731.8	17.72	<0.01
Time	493.9	4	123.47	1.264	
Env. * Time	380.98	4	95.25	0.98	
Error	3058.21	62	97.71		

Figure 4-2. The effect of 42 days of exposure to cold on diastolic blood pressure of chronically cannulated rats. Values are Mean ± S.E.M.



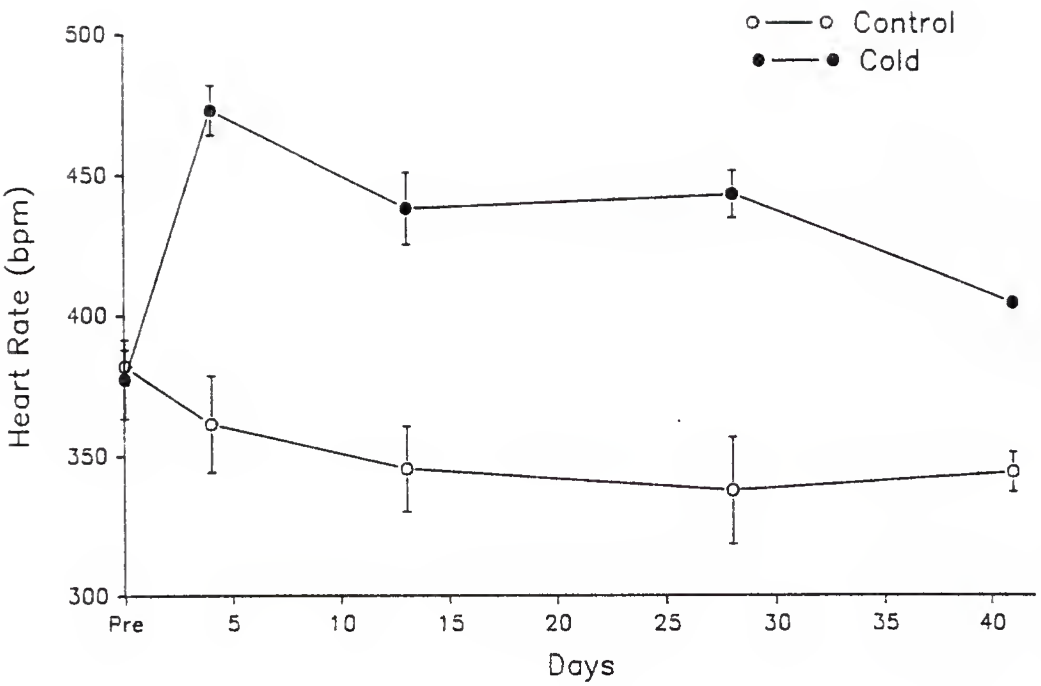
Source	SS	DF	MS	F	P
Environment	5229.48	1	5229.48	61.16	<0.01
Time	1589.67	4	397.42	4.65	<0.05
Env. * Time	1126.87	4	281.72	3.3	<0.05
Error	5301.5	62	85.51		

Figure 4-3. The effect of 42 days of exposure to cold on mean arterial pressure of chronically cannulated rats.
Values are Mean ± S.E.M.
* First point which is significantly ($p < 0.05$) greater than control by Duncan's New Multiple Range test.

Heart rate was significantly increased during exposure to cold as seen by a significant interaction and main effect (Figure 4-4). Heart rate was the highest on day four, with some animals oscillating between 480 to over 500 beats per minute (bpm) during the recording period. Heart rate continued to decline toward pre-cold levels but remained much higher than controls even after 41 days (404 ± 3 vs 344 ± 7 bpm). Similar to previous findings, chronic exposure to cold resulted in significant hypertrophy of the heart, kidneys and adrenal glands (Table 4-1). In addition, the ratio of thyroid/100 g body weight was greater in the cold-treated group, a finding unique to this study. However, when the relationship between body weight (X) and organ weight (Y) was examined by linear regression analysis, only the slope of the relationship between body weight and the weight of the heart was significantly increased (Table 4-2).

Baroreceptor function was assessed throughout the duration of exposure to cold after pharmacological manipulation of blood pressure with the pressor agent, phenylephrine. Baroreceptor function curves were formulated by plotting the mean arterial pressure against the heart period (1/heart rate) at any given moment after bolus i.v. injection of the pressor agent. Typical baroreceptor function curves for both a cold-treated and a control animal prior to, and during exposure to cold are shown in Figure 4-5. The slopes and intercepts of these curves were determined by linear regression and the results submitted to statistical analysis. No significant main effects, either cold or time, nor an interaction was found for the intercept. However, the slope of the relationship between mean arterial pressure (X) and heart period (Y) was significantly depressed during exposure to cold (Figure 4-6). A significant main effect of cold without an effect of time was found.

Chronic exposure to cold significantly ($p<0.05$) increased the concentration of norepinephrine (NE) in plasma (Figure 4-7). The increase in NE is attributed to the low environmental temperature, as only a main effect of cold without a significant main effect of time nor an interaction was evident. The resting or basal concentration of NE was 476 ± 57 pg/ml and when measured after 24 hours of exposure to cold, the



Source	SS	DF	MS	F	P
Environment	80868.83	1	80868.8	75.78	<0.01
Time	13264.35	4	3316.09	3.107	<0.05
Env. * Time	27625.8	4	6906.5	6.472	<0.01
Error	58696.21	55	1067.2		

Figure 4-4. The effect of 42 days of exposure to cold on the heart rate of chronically cannulated rats.
Values are Mean ± S.E.M.

Table 4-1. The effect of chronic exposure to cold on various organ weights of the rat.

organ weight (mg)/(100 g) body weight

	Heart	Kidneys	Adrenal Glands	Thyroid Gland
COLD	338.68 ± 7.24*	966.62 ± 22.30*	17.38 ± 1.81*	6.16 ± 0.32*
CONTROL	285.47 ± 4.31	671.51 ± 26.00	11.92 ± 0.36	5.04 ± 0.31

Values are mean ± S.E.M.
* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.

Table 4-2. The effect of 45 days of exposure to cold on the relationship between body weight (X) and organ weight (Y) in chronically cannulated rats.

$$Y = B(1)X + B(0)$$

	B(1)		B(0)		Correlation Coefficient	
	COLD	CONTROL	COLD	CONTROL	COLD	CONTROL
HEART	4.538*	2.283	-486.20	283.50	0.888	0.902
KIDNEYS	8.329	3.709	561.50	1491.97	0.698	0.476
ADRENALS	-0.335	0.157	214.64	-18.94	0.606	0.833
THYROID	0.059	-0.023	1.11	35.73	0.460	0.361

* Significantly greater than control ($p<0.05$) by independent-sample, one-tailed t-test.

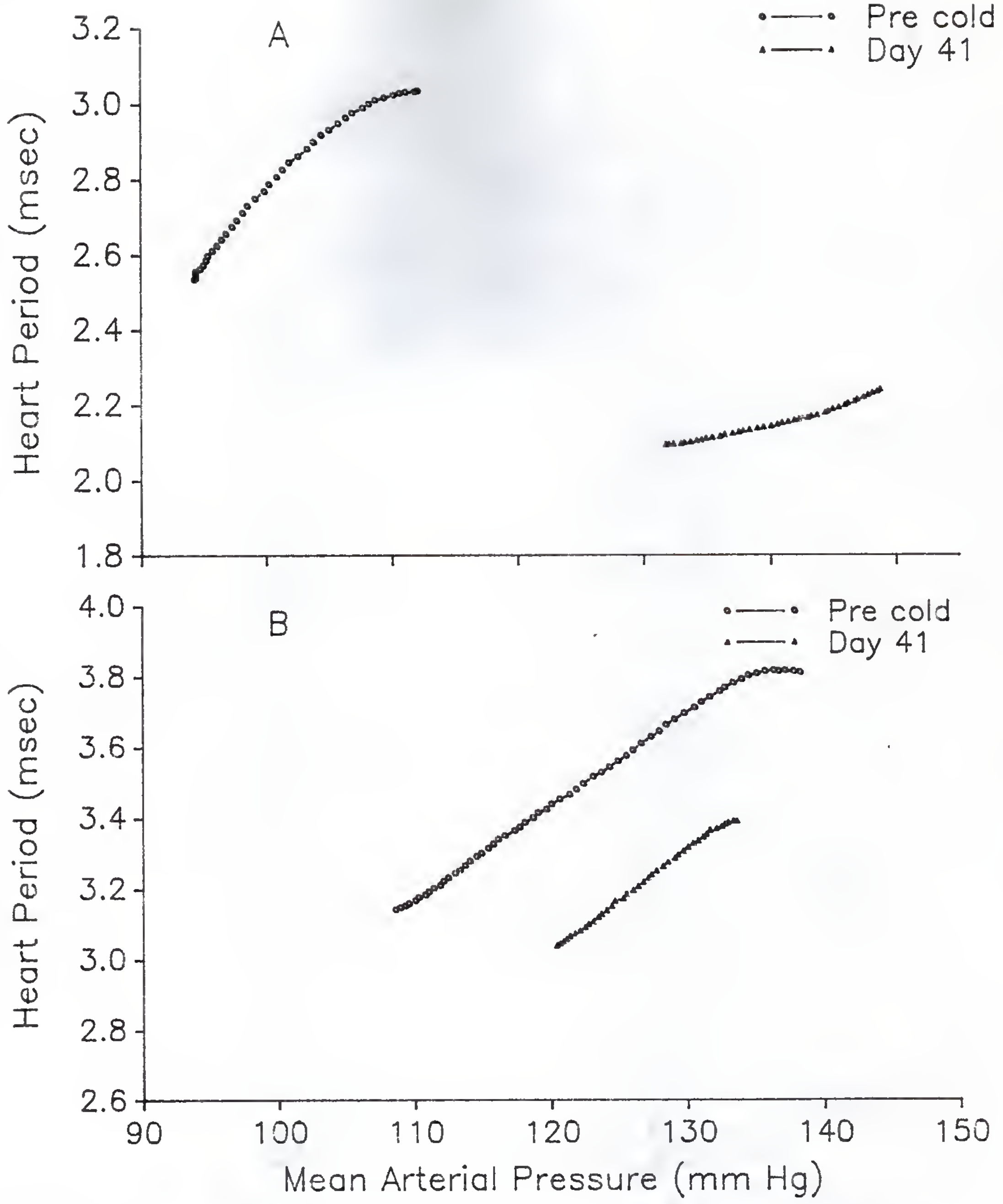
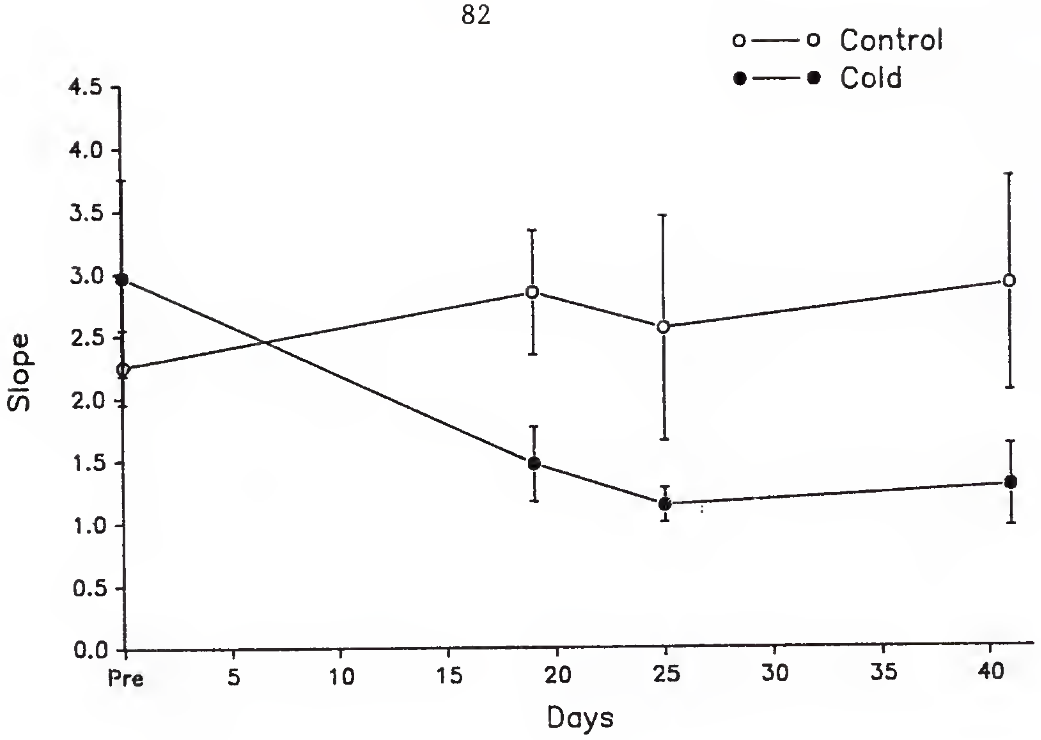
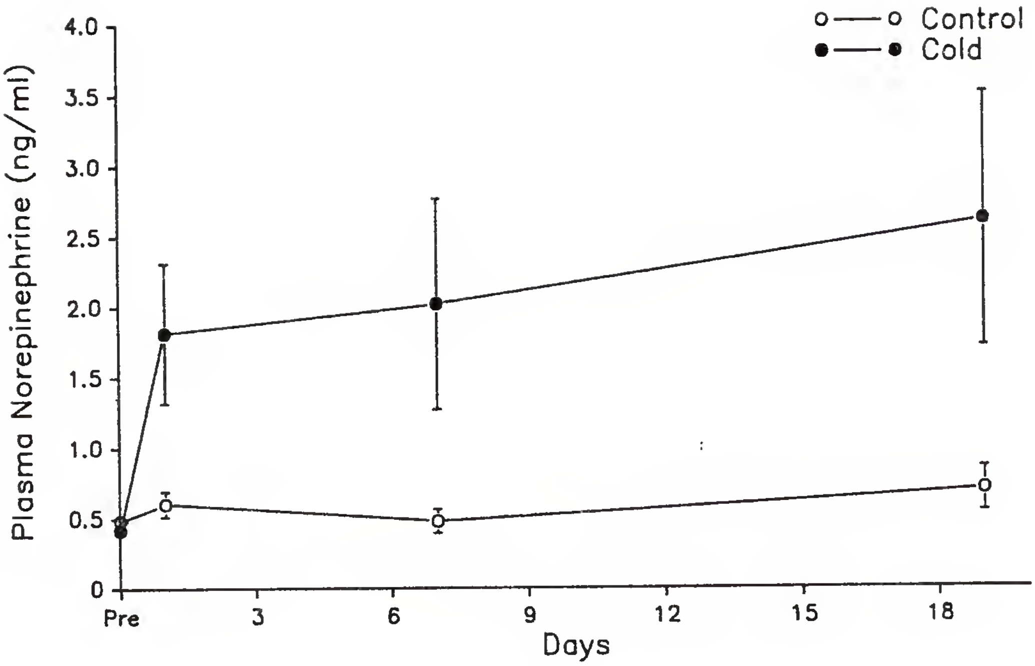


Figure 4-5. The effect of 41 days of exposure to cold on baroreceptor function curves of chronically cannulated rats.
Values are individual (X,Y) pairs of data describing the relationship between mean arterial pressure and heart period (1/heart rate) for any given time, in response to i.v. bolus injection of phenylephrine (2 ug/kg).
A. Baroreceptor function curve of a cold-treated rat prior to, and after 41 days of exposure to cold.
B. Baroreceptor function curve of a control rat prior to exposure to cold and 41 days later.



Source	SS	DF	MS	F	P
Environment	9.176	1	9.176	4.252	<0.05
Time	3.347	3	1.116	0.517	
Env. * Time	9.86	3	3.288	1.524	.22
Error	79.85	37	2.158		

Figure 4-6. The effect of 41 days of exposure to cold on the slope of the baroreceptor function curve in chronically cannulated rats. Values are Mean ± S.E.M.

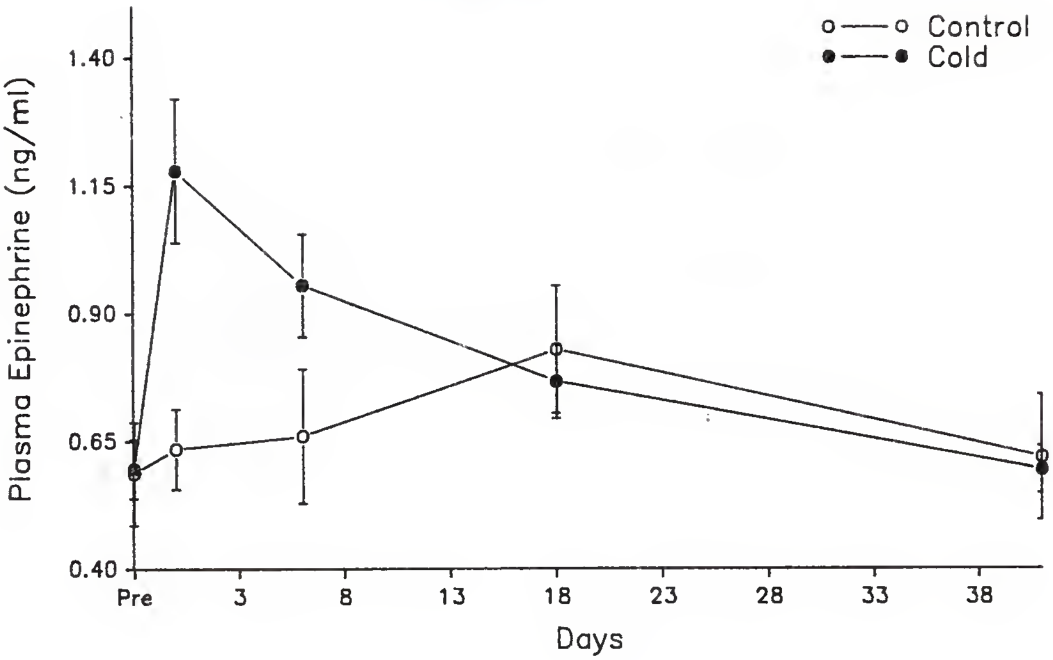


Source	SS	DF	MS	F	P
Environment	19125368.8	1	1912568.8	8.515	<0.01
Time	10981358.3	3	3660452.8	1.63	0.192
Env. * Time	8013216.2	3	2671072.1	1.189	.323
Error	114552360.5	51	2246124.7		

Figure 4-7. The effect of exposure to cold on the concentration of norepinephrine in the plasma of chronically cannulated rats. Values are Mean ± S.E.M.

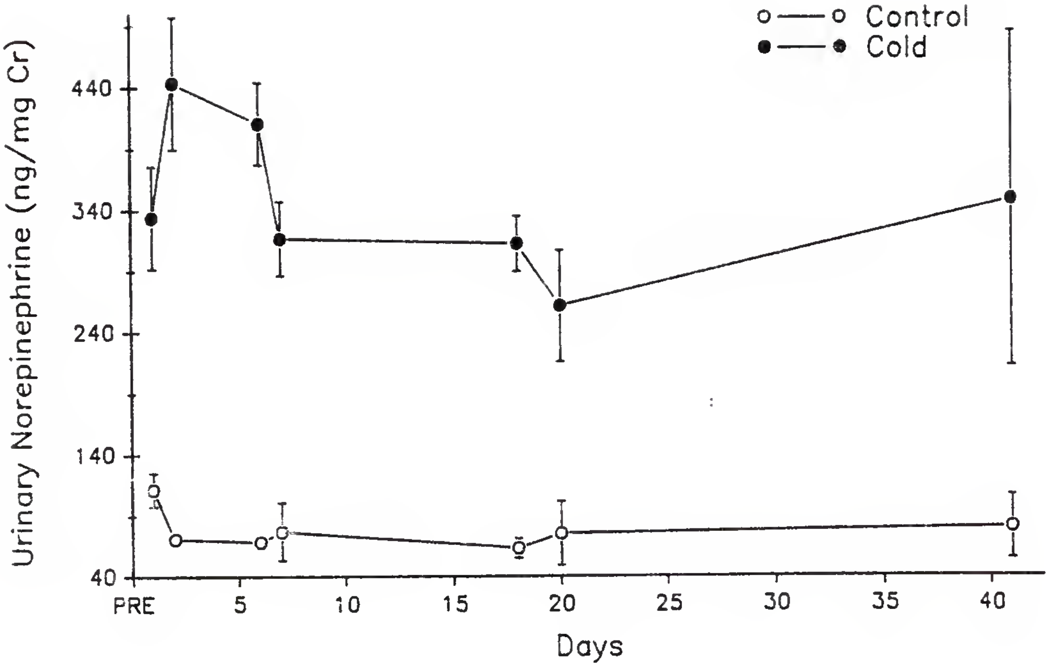
concentration was 1813 ± 904 pg/ml. Although the concentration of NE tended to increase, reaching 2611 ± 950 pg/ml after 19 days of exposure, there was no significant effect of time. The concentration of NE in the plasma remained significantly increased throughout the time it was measured (20 days). The concentration of NE in plasma from the control group remained relatively stable, averaging 565 ± 58 pg/ml over the 20 days measured. Similarly, exposure to cold significantly increased the concentration of epinephrine (E) in the plasma from basal levels of 596 ± 58 to 1179 ± 141 pg/ml (Figure 4-8). In addition to the main effect of cold, there was also a significant effect of time and a significant cold X time interaction. The concentration of E in plasma in the cold-treated group returned to pre-cold levels of 594 ± 47 pg/ml when measured after 19 days of exposure to cold and remained unchanged from control.

The effect of chronic exposure to cold on the urinary concentration of NE, E, and dopamine (DA) is shown in Figures 4-9, 4-10, 4-11. Urinary catecholamine data were expressed as ng/mg creatinine (Cr)/day, in an effort to adjust for changes which may occur in renal activity. Norepinephrine, E and DA were all significantly increased by exposure to cold as indicated by a significant cold X time interaction. However, the rate at which each catecholamine returned toward control or basal levels differed. The concentration of NE in the urine of the cold-treated group increased by the first day of exposure and did not return to control levels even after 41 days of exposure to cold (Figure 4-9). In general, a large amount of variability was evident within the cold-treated group. In contrast, the urinary concentration of NE of the control group remained relatively constant when expressed as ng/mg Cr. The urinary output of epinephrine was elevated in the cold-treated group only on the second day of exposure. Although higher than control levels at many times during the study, the intragroup variability for both cold-treated and control was very large. As with epinephrine, the urinary concentration of dopamine was significantly increased as indicated by a significant cold X time interaction. In addition, intragroup variability was also very large.



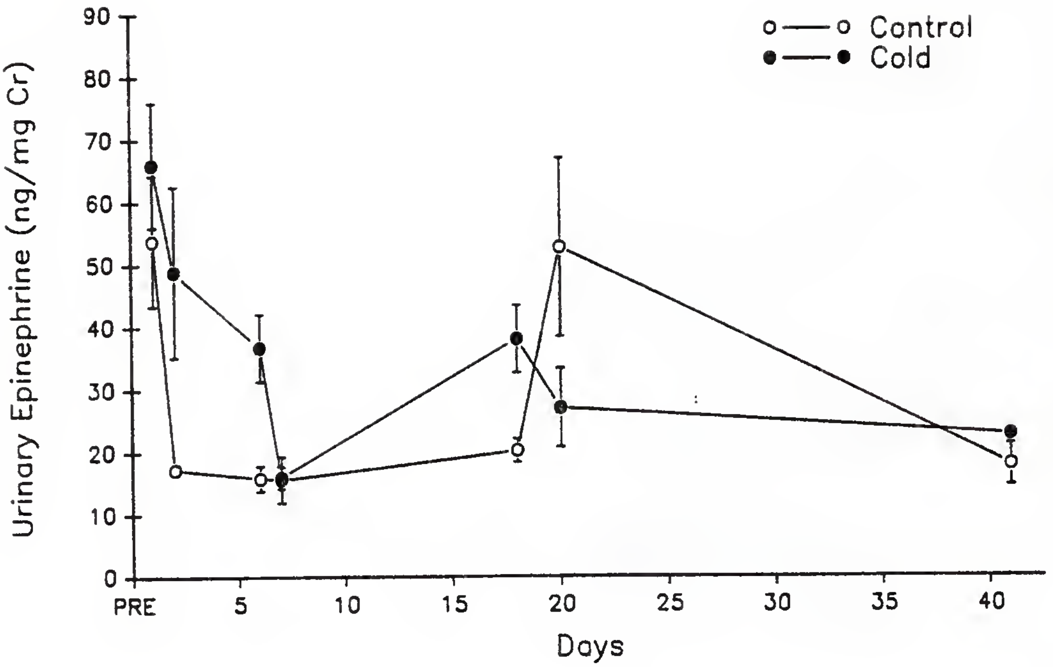
Source	SS	DF	MS	F	P
Environment	416846.8	1	416846.8	4.499	<0.05
Time	1094352.8	4	273588.2	2.953	0.026
Env. * Time	979958.3	4	244989.6	2.644	0.04
Error	6115219.2	66	92654.8		

Figure 4-8. The effect of 41 days of exposure to cold on the concentration of epinephrine in plasma from chronically cannulated rats. Values are Mean ± S.E.M.



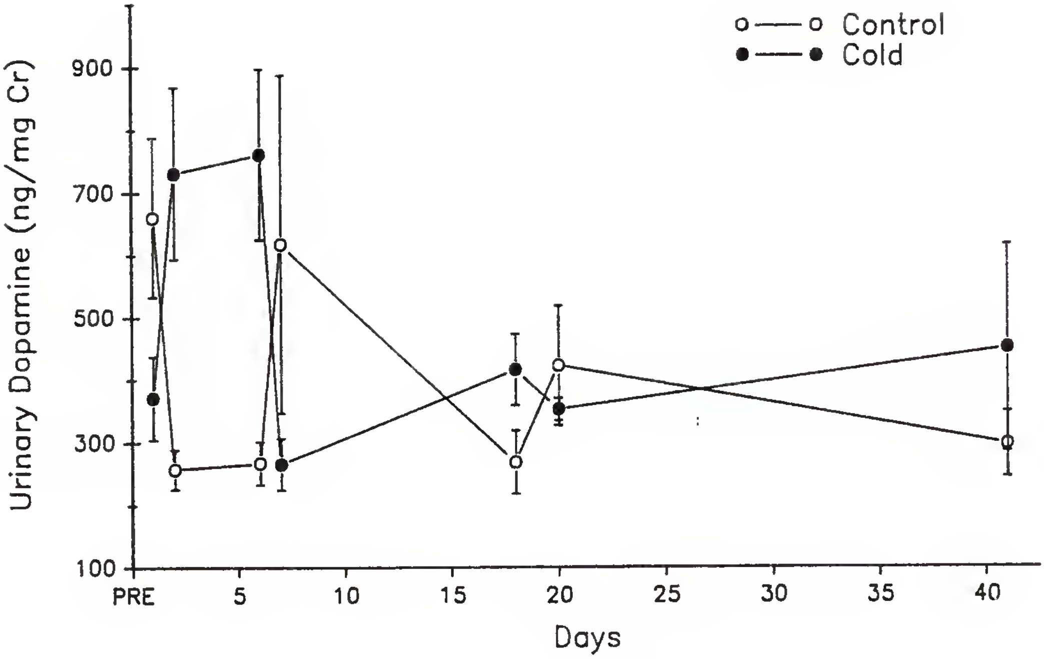
Source	SS	DF	MS	F	P
Environment	100484274.6	1	100484274.7	130.0	<0.01
Time	27176949.1	7	3882421.3	5.02	<0.01
Env.* Time	16114161.2	7	2302023.0	2.98	<0.01
Error	85795610.3	111	772933.4		

Figure 4-9. The effect of 42 days of exposure to cold on the output of norepinephrine in the urine.
Values are Mean ± S.E.M



Source	SS	DF	MS	F	P
Environment	53947.2	1	53947.2	.422	
Time	4992838.9	7	713262.7	5.58	<0.01
Env. * Time	2491191.8	7	355884.6	2.78	<0.05
Error	12792677.8	100	127926.8		

Figure 4-10. The effect of 42 days of exposure to cold on the output of epinephrine in the urine.
Values are Mean ± S.E.M.



Source	SS	DF	MS	F	P
Environment	396114.3	1	396114.3	.119	
Time	127723031	7	18246147	5.48	<0.01
Env. * Time	79174773	7	11310682	3.4	<0.01
Error	369761638	111	3331186		

Figure 4-11. The effect of 42 days of exposure to cold on the output of dopamine in the urine.
Values are Mean ± S.E.M.

In an effort to determine whether the concentrations of catecholamines in the urine were a good index of the concentration of catecholamines in the plasma, linear regressions were performed on plasma (X) versus urinary concentration (Y) of NE and E. Data from urines collected for either 24 hours prior to, or 24 hours after the blood sample was taken were compared against the plasma value. No significant correlation could be found between the concentration of plasma (pg/ml) and urine catecholamines (ng/mg Cr). Further, the correlation coefficients were poor, ($r < 0.25$) for all relationships. In an effort to determine whether expressing the urinary catecholamines as a function of creatinine was contributing to the poor correlation, regression analyses were rerun on plasma (pg/ml) versus urinary concentration expressed as total ng/day. No difference was found between the two analyses, i.e. whether the concentration of catecholamines in urine was expressed as ng/mg Cr or as total ng/day. The relationship between these two measurements is shown for norepinephrine and epinephrine in Figures 4-12 and 4-13.

Discussion

The present study is the first to utilize chronic arterial cannulation to measure blood pressure, baroreceptor function and the activity of the sympathetic nervous system (SNS) during exposure to low environmental temperature. In previous experiments, chronic exposure to cold resulted in a significant elevation of systolic blood pressure, as assessed indirectly utilizing the tail cuff technique, and cardiac hypertrophy. Similarly, in the present study, exposure to cold resulted in a significant elevation of systolic blood pressure (Figure 4-1, Table 4-2) and cardiac hypertrophy. After 42 days of exposure to cold, SBP increased to 152.5 ± 4.0 mm Hg (Figure 4-1). The magnitude of this increase is quite similar to that seen using the indirect tail technique for measuring blood pressure after a similar length of exposure (Chapter Two). Additionally, diastolic and mean arterial pressures as well as heart rate were significantly increased (Figures 4-2, 4-3, 4-4). This is an important finding as systolic blood pressure is primarily a function of cardiac output, while diastolic blood pressure is indicative of a change in vascular

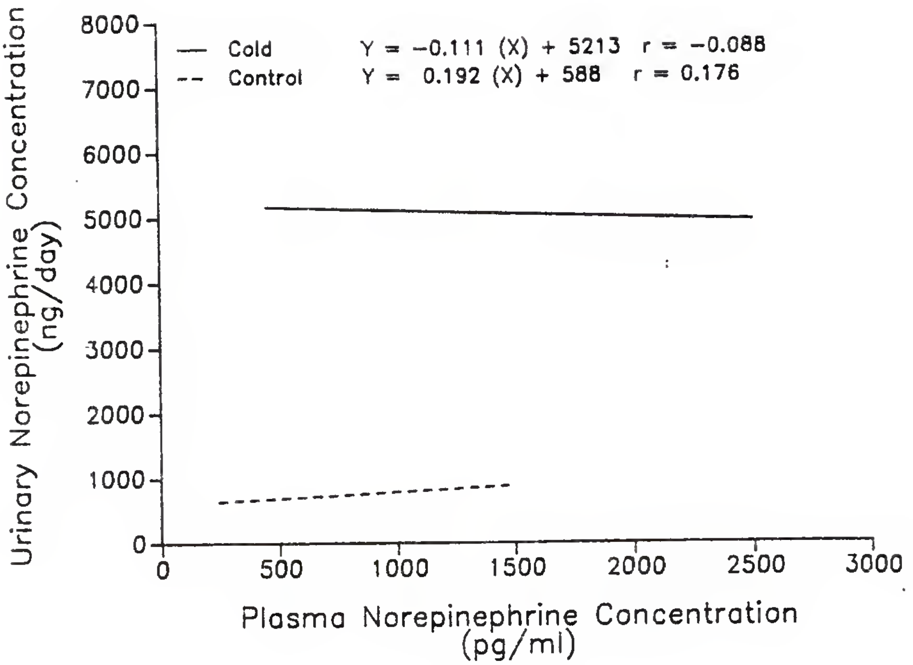


Figure 4-12. The relationship between the concentration of norepinephrine in the plasma and urine of chronically cannulated control and cold-exposed rats as determined by linear regression analysis.

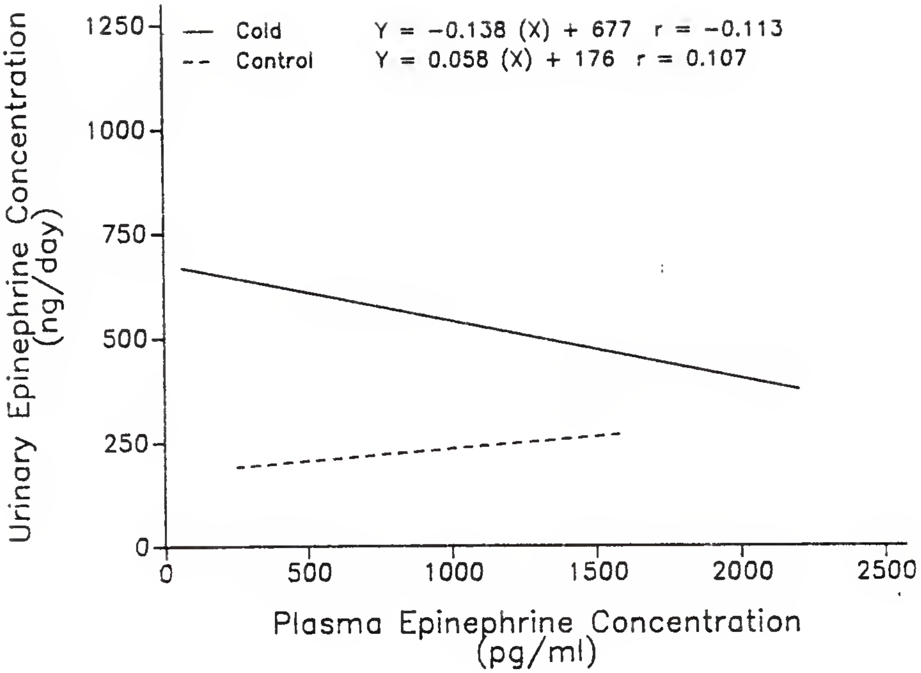


Figure 4-13. The relationship between the concentration of epinephrine in the plasma and urine of chronically cannulated control and cold-exposed rats, as determined by linear regression analysis.

resistance. An increase in vascular resistance is a characteristic of hypertension. In the present study, diastolic blood pressure increased to 103.0 ± 2.8 mm Hg and was accompanied by an increase in mean arterial pressure to 124 ± 2 mm Hg. The significant interaction demonstrated by each of these pressures indicates the slopes of the cold-treated group are different from control. This suggests that the pressures had not peaked at this time and may have continued to increase. It also suggests, that changes which contributed to the elevation of blood pressure may also be incomplete and perhaps were still occurring, i.e. a steady state had not been achieved. Whether blood pressure would continue to increase in this manner, and for how long, is undetermined. The finding of increased systolic, diastolic and mean blood pressures in combination with cardiac hypertrophy support the hypothesis as initially proposed by Fregly et al. (61) that chronic exposure to cold results in the development of hypertensive disease in rats.

It is interesting, that the 20 mm Hg increase in mean arterial pressure seen in the present study is quite similar to the rise in mean arterial pressure reported by Reed et al. (162) in young men (21± 2 years) stationed in Antarctica. In this particular study, the mean arterial pressure rose significantly from a baseline of 85 to 101 ± 3 mm Hg over a 42 week period of extended residence. It appears that the study by Reed et al. (162) is the only published work in which the effect of long-term (although intermittent) exposure to cold on blood pressure in humans has been measured. However, the idea that low environmental temperature adversely influences blood pressure of humans is not a new one, as several reports exist within the literature. Prineas et al. (157) measured the resting blood pressure of 10,000 Minneapolis school children over a 4 month period in effort to establish norms for this population. Even after extensive efforts to eliminate methodological biases, these authors (157) reported a significant effect of season on blood pressure, with winter (January and February) diastolic pressure more than 3 mm Hg greater than the pressure recorded in the spring (April). Similarly, when blood pressures which, were recorded during the British Medical Research Council's treatment trial for mild hypertension, were analyzed on the basis of season, Brennan et al. (17) found a significant seasonal influence. For each age, sex and treatment, these authors (17) found greater systolic and diastolic blood pressures in the winter as compared to the summer. The initial impetus for the analysis of a seasonal influence on blood pressure stems from earlier reports which demonstrated a much higher mortality rate from ischemic heart disease and stroke during the winter in England and Wales. In a later study, this same relationship (increased mortality with decreasing temperature) was seen in New York, England and Wales (20). The high correlation coefficients lead Bull (20) to suggest a causal relationship between temperature and mortality. In a recent study by Hata et al. (83), the same seasonal variation in blood pressure was seen in essential hypertensive but not normotensive patients. The above reports suggest strongly, that cold-induced hypertension may not be unique to the rat.

An important finding in the present study, in which blood pressure was measured directly from an indwelling cannula, is the time-course or onset of the increased blood pressures. Systolic blood pressure was significantly ($p< 0.05$) increased by the fourth day of exposure to cold, the first time at which it was measured. Mean arterial pressure was significantly increased after 13 days of exposure to cold. Previously, the first significant elevation of systolic blood pressure was evident after 21 days of exposure to cold (Chapter Two), nearly two weeks later than reported in the present study.

There are several possible explanations for the difference in the onset of the elevation of blood pressure between the two techniques. First, in order to measure indirect blood pressure, the animals must be restrained and warmed at 37°C for approximately 15 minutes. Restraint represents a very potent stress for rats. Visually, the animals settle down over time, or they appear to adapt to the general stress of restraint. The observation of adaptation to restraint prompted Popovic (156) to examine this phenomenon. When the concentration of ACTH and corticosterone (B) were measured in restraint-adapted animals after an acute episode of restraint, both ACTH and B were significantly increased above prerestraint and controls levels (156). These results refute the idea of adaptation to restraint in rats (156). In addition to ACTH and B, the catecholamines are very sensitive to general stressors and would also be expected to increase with restraint. Since the concentration of NE and the activity of the SNS are already increased in the cold-treated animals, it is possible that restraint results in a "relatively greater" response in the control group. In essence, the delta or difference from baseline in the control group may be greater. Resting SBP of restrained controls (Chapter Two) ranged between 115 to 142 mm Hg while cannulated controls ranged between 110 and 120 mm Hg. In addition, the intra-group variability was much greater in the restrained controls. Thus, although the cold-treated animals exhibited an increase in SBP, the results were not significant until they reached a much higher absolute value.

A second factor is inherent in the measurement of systolic blood pressure. Systolic blood pressure is often a function of cardiac output. The cardiac output of cold-treated animals is very high while the animal is in the cold (89,91). An increased cardiac output is necessary to supply sufficient substrate to the tissues, and meet the increased demands for heat production. When the cold-treated animal is removed into a previously thermoneutral environment (26°C), and then subjected to a mild heat stress (warmed at 37°C for 15 minutes), cardiac output, and thus systolic blood pressure, may change (decrease) rapidly. Thus, systolic blood pressure may decrease toward control levels. Even a small decrease in the cold-treated group, when combined with a slightly higher reading in the control group due to restraint, could contribute to the lack of difference seen between the two groups and thus the difference between the two techniques. How the cold-adapted animal responds to a heat stress and what effect removal from cold has on systolic blood pressure was not determined in these studies.

Regardless of the technique used to measure blood pressure, it can be concluded that chronic exposure to cold results in a sustained elevation of systolic, diastolic and mean arterial pressures, an increased heart rate and cardiac hypertrophy, i.e. fundamental characteristics of hypertensive disease. The onset is fairly rapid, occurring within two weeks, while the magnitude of the sustained increase in blood pressure reached 150 mm Hg with approximately 45 days of exposure. It can be concluded that chronic exposure to cold represents a unique method for inducing hypertension in rats in which both the onset and magnitude of increased blood pressure is repeatable.

In the present study, baroreceptor reflex function was assessed throughout the exposure to cold by pharmacologically manipulating blood pressure with phenylephrine. The relationship between mean arterial pressure and heart period (1/ heart rate) after the administration (i.v.) of phenylephrine was quantified. The increase in blood pressure was dramatic and was followed by a rapid return to control levels. Utilizing a scientific software package (Assystant Plus 4.0), the mean arterial pressure (peak to baseline) (X) was regressed against the heart period (peak to baseline) (Y) at any instant. The linear portion of the resultant curve was utilized to determine the activity of the reflex. The slope and intercept derived for each rat was used as an indication of the sensitivity or gain of the reflex and setpoint, respectively (84). The slopes from the cold-treated group were significantly decreased during exposure to cold, while no change in the intercepts was apparent. Thus, the sensitivity of the baroreceptor reflex was depressed, without a change in the setpoint. The significant cold X time interaction seen for the slopes of the cold-treated group suggests that the sensitivity may still be decreasing. This would agree with the positive slope seen for the blood pressure curves of the cold-treated group suggesting a steady state has yet to be attained.

The arterial baroreceptor reflex functions to control arterial pressure via a negative feedback system. Stimulation of this system, by an increase in arterial pressure, reflexly mediates a decreased activation of the SNS. The baroreceptors are considered to be the first line of defense in maintaining a normal arterial pressure. In addition, there is substantial evidence to suggest that the baroreceptors are rapidly reset, possibly within 5 to 20 minutes of a sustained change in pressure (114). If the baroreceptor system is reset to a higher level, a decrease in the error signal to the regulatory centers will result. After resetting, the baroreceptor system will act to control arterial pressure at this new, higher pressure. In addition, a decrease in the sensitivity of the baroreceptor reflex would suggest a decrease in the negative feedback signal, and thus a decrease in the reflex-mediated compensatory changes.

The decreased baroreceptor sensitivity reported in the cold-induced model of hypertension is in agreement with the decreased sensitivity seen in various other forms of hypertension including human essential hypertension (67), Dahl salt-sensitive rats (68), spontaneously hypertensive rats (102, renal hypertensive rabbits (74), and DOCA-salt rats (140). Most of the above studies examined baroreceptor function after the development of hypertension. This offers little information with regard to a role of the baroreceptor system in inducing hypertension. However, Nakamura et al. (140) followed changes in baroreceptor function during the development of DOCA-salt hypertension. These authors reported a centrally mediated attenuation of the baroreceptor reflex in prehypertensive animals and suggested this change as a contributory factor in the DOCA-salt model (140). The loss of the baroreceptor reflex, and thus the negative feedback and activation of the aortic depressor nerve, may represent a mechanism for inducing hypertension. Kline et al. (112) have induced hypertension with aortic depressor nerve transection in rats. In the present studies, the sensitivity of the baroreceptor reflex was depressed during exposure to cold. However, insufficient data were available to assess baroreceptor function during the initial phase of exposure to cold. Complete data were available in the cold-treated group only after 19 days of exposure; well after a sustained elevation of mean arterial pressure had occurred. This leaves the question of the role of the baroreceptor reflex in this model unanswered. In addition, only the ascending limb of the reflex was measured. Future studies will be required to assess the response of the baroreceptor reflex to a hypotensive challenge and during the initial exposure. Perhaps by closely examining the time-course relationship between the changes in sensitivity of the baroreceptor reflex and increased arterial pressure, it will be possible to determine whether the baroreceptor system contributes to, or is a result of, cold-induced hypertension.

In the present study, systolic blood pressure was increased after four days of exposure to cold; much earlier than previously reported (17-23 days). This earlier onset supports the hypothesis that cold-induced hypertension has a large neurogenic component. This neurogenic component may be attributed to the overactivation of the SNS, which is indicated by increased urinary concentration of catecholamines (119,133,122). In the present study, the concentrations of NE and E in both the plasma and urine were significantly increased by exposure to cold. This finding is in agreement with numerous other studies which have reported an increased urinary concentration of catecholamines in a variety of animals exposed to cold (133,196,120,121,158,6,183). The concentration of NE remained significantly increased in both plasma and urine at 20 days, and in the urine after 41 days of exposure. No data were available for the concentration of NE in plasma at 42 days of exposure to cold, due to the decreased number of animals with functioning cannulae in the cold-treated group, and a loss of the NE peak on the chromatograph due to an undetermined interference. However, the sustained elevation of NE is in agreement with a sustained activation of the SNS during chronic exposure to cold (134). In contrast to NE, the concentration of epinephrine in plasma rapidly returned to control or baseline values within 20 days of exposure. This return to baseline occurred earlier than previously reported by Leduc (122). The urinary concentrations of both E and dopamine (DA) exhibited large variations both within and between subjects. There is no apparent reason for this inconsistency.

The different patterns exhibited by NE and E strongly support the idea that NE is the major stimulus for nonshivering thermogenesis which must be sustained for survival, while E functions primarily in a reserved capacity. The fact that the concentrations of epinephrine in the plasma of cold-treated animals quickly returned to control levels suggests that a) the adrenal medulla responded to the initial exposure to cold; b) the mechanism(s) inducing this response was short-lived; or c) the cellular responsiveness to E increased, thus less E was required. Leduc (122) examined the possibility of depletion of the catecholamine storage in the adrenal medulla. He found a slight decrease in the content of E in the adrenal medulla on the first day of exposure to cold, but he also demonstrated a rapid resynthesis and storage in excess of controls at any time thereafter. Thus, no change in the content of E in the adrenal medulla was found.

In an effort to determine whether the concentration of catecholamines in the urine was a reliable index of their concentration in plasma, correlations were performed on the plasma (X) versus urine (Y) concentrations of both NE and E. The results indicated a poor relationship ($r < 0.25$) between these two variables. Thus, in spite of similar trends between these two variables, mean urine values increased when mean plasma values increased, urinary catecholamines were not a good index of resting plasma catecholamine concentrations. This is not really a surprising result. Plasma catecholamines represent an instantaneous or brief glimpse at what the catecholamine profile is throughout the entire day. Ideally, by collecting at a similar time, from a resting animal, it is an index of the activity of the SNS and the adrenal medulla in the resting state. Urinary catecholamines represent the entire collection period and have been used by many investigators as an indication of the activation of the SNS. However, urinary catecholamines are affected not only by the concentration of catecholamines in the blood, but also by their rate of clearance. In addition to the numerous factors which may affect the clearance of substances from the blood, renal nerve stimulation has been shown to have a direct affect on the amount of NE excreted into the urine (116). In a model characterized by a hyperactive SNS, it is possible that the activity of the renal nerve is similarly increased. Additionally, the initial hypothesis was that NE was only filtered by the kidney (148), recently however, Lappe (116) demonstrated that the kidney not only filters but secretes NE. The work of Kuchel et al. (15) provide an additional explanation for the poor correlation between plasma and urinary catecholamine concentrations. These authors reported an increased renal clearance of NE in labile (borderline) hypertensives. If an increased blood pressure or the lability of pressure alters the clearance of NE and possibly E by the kidney, then urinary output of catecholamines is an inappropriate means for assessing activation of the SNS in hypertension. In the present study, only the amount of free catecholamine was measured, none of the metabolites nor the glucuronidated or sulfoconjugated forms were measured. It is very possible that the clearance of catecholamines from the blood is altered during chronic exposure to cold by nonrenal mechanisms; for instance, either increased reuptake or increased conversion to metabolites. Measurements of urinary catecholamines would not reflect such alterations. Although not a sensitive index of the resting concentration of catecholamines in the blood for any particular moment, urinary catecholamines represent a simple, noninvasive means to determine major trends or perhaps an effect of a particular treatment.

The purpose of measuring both plasma and urinary concentrations of catecholamine was to determine whether the SNS remained hyperactive during chronic exposure to cold. If the assumption that the concentration of NE in the plasma is a direct result of spillover from both central and peripheral nerve terminals is valid, then the results support such a conclusion. Further, there is no indication of a decreased activity or decreased concentration of NE in the plasma. As NE is the stimulus for nonshivering thermogenesis, a sustained increased would be anticipated.

The logical question which arises is what role does the increased activity of the SNS play in cold-induced hypertension? Unfortunately, it is not possible to determine whether the circulating NE is derived from central or peripheral sympathetic nerves. Nor is it possible to determine the region of origin, i.e. NE released at thermogenic sites versus NE released at vascular smooth muscle receptors versus that released from the adrenal medulla. The differential distribution or activation of the peripheral sympathetic nervous system represents a major problem in interpreting these results. Further, it is entirely possible to have an increased neuronal release and a decreased responsiveness due to changes either at the receptor or beyond the receptor. Unless plasma and urinary concentrations, as well as target responsiveness are assessed simultaneously, the role of the SNS in this and other models of hypertension will remain elusive.

Similar difficulties are present when attempting to interpret the catecholamine data from the various human studies which compared hypertensive with normotensive patients, as well as from the experimental models of hypertension. In a review on the sympathetic nervous system and hypertension, De Champlain (39) summarized the results as inconclusive, exhibiting increased, decreased and unchanged levels of urinary catecholamines. De Champlain (39) suggested that a large amount of the variability in the results from studies which measured urinary catecholamines is inherent due to the heterogeneity of the disease. Still, some interesting generalities can be formulated. Urinary excretion of catecholamines fail to indicate a hyperactivation of the SNS, as their concentration or output has been reported to be increased, decreased or unchanged (39). Gitlow et al. (65) failed to detect any abnormalities in either turnover, storage or reuptake in most essential hypertensives. Concentrations of NE in plasma are a much better indicator of SNS activation, although the results from hypertensive patients are inconclusive. Louis et al. (131) reported a positive correlation between concentrations of NE in plasma and diastolic blood pressure in hypertensive patients, while these same authors later reported normal concentrations of NE in 41% of essential hypertensive patients they studied (132).

A possible explanation for the discrepancies between the concentrations of NE measured in plasma and the apparent degree of vasoconstriction (diastolic blood pressure), could be due to compensatory mechanisms invoked in an effort to maintain a normal blood pressure. For instance, a decreased receptor number, decreased vascular responsiveness, or a change in blood volume could all result in a normalization of blood pressure in the presence of an increased circulating concentration of NE. Changes in vascular reactivity have been demonstrated in numerous models of experimental hypertension including: SHR (49), renal (110), human hypertension (39), as well as in rats chronically exposed to cold. Bryar et al. (19) reported a decreased responsiveness to alpha-adrenergic agonists in aortic rings of cold-adapted rats in vitro. A decreased reactivity in the presence of an increased agonist could result in a normal diastolic pressure. Thus there is a poor correlation between the concentration in plasma and the response (resting diastolic blood pressure). A decreased vascular responsiveness may be one mechanism used by the cold-treated animal to neutralize the vasoconstrictor property normally associated with an increased concentration of the circulating ligand, NE.

It is difficult to determine the exact contribution of the SNS to the elevated arterial pressure seen with chronic exposure to cold. In addition to a direct vasoconstrictor effect, a hyperactive SNS could increase blood pressure through a variety of indirect mechanisms. An increased activity of the SNS could alter renal function, or may increase plasma renin activity and concentrations of AII in plasma. Angiotensin II is a very potent vasoconstrictor agent. Further, the exact mechanisms which result in the increased activity of the SNS remain for investigation. It is quite possible that changes within the central nervous system have occurred, and that these changes account for the increased output from the SNS. Preliminary studies from this laboratory have reported a increased specific binding of alpha$_2$-adrenoceptors and increased affinity of alpha$_1$-adrenoceptors in the hypothalamus-thalamus-septal region, an important region for controlling both body temperature and blood pressure (151). Whether changes in the CNS are the result of the cold-induced hypertension or a contributory mechanism remains to be investigated. In addition, the mechanisms by which an increased activity of the SNS induces or sustains the hypertension in this model remains unclear. It appears that cold-induced hypertension, like human essential hypertension is a complex, multifactorial syndrome.

CHAPTER FIVE
THE ROLE OF VASOACTIVE HORMONES IN COLD-INDUCED HYPERTENSION

Introduction

There is sufficient evidence to suggest that chronic exposure to cold induces hypertension in rats (Chapters Two-Four). This model of hypertension is very repeatable with regard to both the onset and magnitude of the elevation of blood pressure. Chronic exposure to cold also results in cardiac hypertrophy, another characteristic of hypertension. There is also sufficient evidence to suggest that the cold-induced model of hypertension has a large neurogenic component. Chronic exposure to cold results in an increased activity of the sympathetic nervous system (SNS). This increased activity is necessitated by the demand to both decrease heat loss (vasoconstriction and piloerection) and to increase heat production (nonshivering thermogenesis), which maintain body temperature. These mechanisms regulating heat loss and heat production are mediated mainly by the SNS. Throughout the duration of exposure to cold, the concentration of norepinephrine (NE), the neurotransmitter of the SNS, is significantly increased in the plasma and urine. Although impossible to determine the exact origin of the circulating NE, it is assumed to result from spillover from sympathetic nerve terminals.

It does not appear that cold-induced hypertension is simply the result of an increased stimulation by the SNS directly at the vasculature. If this were the case, an immediate and sustained increase in arterial pressure should occur. As previously reported (Chapter Four), systolic pressure is increased by the fourth day of exposure, while diastolic and mean arterial pressures are not increased above baseline until after two weeks of exposure to cold. In addition, Fregly et al. (60) and Bryar et al. (19)

measured the alpha-adrenergic (vasoconstrictor) responsiveness of aortic smooth muscle rings in vitro and reported a decreased responsiveness in the cold-treated (5-6 weeks at 6°C) group. The desensitized vasculature response was specific to NE as the response to other constrictor agents was unchanged. The presence of a desensitized, or down-regulated receptor may effectively negate, or at least in part diminish, the increased activation of the SNS. This altered vascular sensitivity may represent a compensatory mechanism by which the animal attempts to maintain a normal blood pressure.

There are numerous mechanisms by which a hyperactive SNS may contribute to an increased peripheral vascular resistance other than direct vasoconstriction. An increase in the activity of SNS stimulates the release of the enzyme, renin, from the kidney. Renin converts angiotensinogen to angiotensin II (AII). Angiotensin II is one of the most potent vasoconstrictor agents produced physiologically. Thus, an increase in the circulating levels of AII may also act directly at the vasculature to contribute to cold-induced hypertension. Angiotensin II also has a direct effect (independent of aldosterone) on the kidney to induce sodium and water retention (40). Additionally, AII stimulates the release of aldosterone. Aldosterone increases sodium reabsorption. The combination of an increased AII and aldosterone concentration act to increase sodium and fluid retention, and thus arterial pressure.

Angiotensin II is only one hormone which may be produced in increased amounts during chronic exposure to cold. The glucocorticoids play an important role both in maintaining body temperature and in mobilizing substrates for energy metabolism (57). Acute exposure to cold results in a large increase in glucocorticoid secretion in a variety of animals, including humans (93,168,180). In the case of rats, exposure to cold results in elevated concentrations of both corticosterone and ACTH in plasma (192). With continued exposure however, the concentrations of both ACTH and corticosterone in plasma return to baseline. ACTH remained elevated for 1 week during exposure to cold, while corticosterone returned to baseline within 2 weeks (192,119). The work of Scoggins et al. (171) suggests a role for ACTH and adrenocortical steroids in the induction of hypertension in sheep and in humans. In addition, each of these hormones may interact and possibly potentiate the effects of NE by acting as either neuromodulators or by altering the responsiveness at the level of the receptor for NE.

Another hormone which is a very potent vasoconstrictor agent in physiological concentration is vasopressin (AVP). The role of this hormone in the control of the cardiovascular system has often been overshadowed by the its more familiar role in fluid balance. Circulating AVP has been shown to potentiate the sensitivity of the baroreceptor reflex (31,35,7,189), interact with the CNS to modify reflex control of heart rate (189,35), and vascular resistance (30,189,35). The work of several investigators suggests that AVP may play an important role in the development of hypertension (30,139,138). Until recently, it was very difficult to determine the concentration of AVP in the plasma. Whether the concentration of AVP in the blood is affected by chronic exposure to cold is unknown.

Whether the SNS acts singularly, or interacts with a variety of vasoconstrictor agents, to increase blood pressure is unknown. The purpose of these experiments was to describe the hormonal profile of animals chronically exposed to cold, in efforts to determine whether they may be involved in cold-induced hypertension.

Methods

Male rats of the Sprague Dawley strain initially weighing 200-250 g were used. All animals were housed in individual cages and provided powdered Purina Laboratory Chow (#5001) and tap water ad libitum. The vivarium was maintained at 26°C and on a 12:12 light:dark cycle. Two weeks were allowed for the animals to adapt to their new environment. The animals used in this experiment are the same ones that were used in Chapter Four. All animals were prepared with chronic femoral arterial and venous cannulae, as described previously (Chapter Four). Ten days were permitted for the animals to recover fully from the effects of surgery. On the first experimental day, all animals were moved into temperature controlled, environmental chambers (either 26° or 6°C) as described previously. At no time during the experiment were the animals removed from their respective environments. Blood pressures were measured as previously described (Chapter Four).

Sample Collection

Arterial blood samples (< 5 ml/kg) were collected at various times throughout the experiment as described previously (Chapter Four). After the samples were spun, the plasma was removed and placed into labeled polypropylene tubes and stored at -80°C until analyzed for plasma renin activity and concentration of aldosterone. The hematocrit data were recorded; the plasma was removed and stored in micro-tubes for later determination of its corticosterone, sodium, and potassium concentrations.

In addition to the samples collected from chronically cannulated animals, blood, which had been collected from the trunk at the time of sacrifice from each of the previous experiments, was also analyzed. Aliquots of plasma were stored at -80°C until analyzed. The concentrations of aldosterone, ACTH, arginine vasopressin, corticosterone and plasma renin activity were measured in samples of plasma from cold-treated and control rats.

Measurements

Arginine vasopressin

Arginine vasopressin (AVP) was extracted from thawed samples of plasma using a bentonite extraction procedure (Appendix B). The concentration of AVP in the plasma was determined by radioimmunoassay. Briefly, 100 ul of either concentrated (extracted) sample or standard (Sigma), 25 ul AVP antibody (Amersham, Arlington Hts, IL), and 25 ul ^{125}I-AVP (Amersham) were incubated at 4°C for 24 hours. The free ligand was separated by the addition of dextran-coated charcoal and centrifuged at 2,000 x g for 15 minutes. The supernatant was aspirated and the remaining charcoal pellet, which contained the free AVP, was counted in a Beckman 5500 gamma counter for 4 minutes. Values were expressed as pg AVP/ ml. The complete method appears in Appendix B.

Plasma renin activity

Plasma renin activity was assessed using a micromodification of the technique originally described by Page et al. (149) and currently in use in this laboratory. Briefly, angiotensin I (AI) was generated from 250 ul samples of plasma. The amount of AI generated was determined by radioimmunoassay. The complete assay, extraction procedure and precision appear in Appendix C.

Aldosterone

The concentration of aldosterone in plasma was measured in unextracted samples by radioimmunoassay. The gamma-coat kit (Diagnostic Products Co., Los Angeles, CA) has a detection limit of 25 pg/ml. Coefficient of variability for intraassay measurements was 3-8 % and interassay variability was 4-10%.

Corticosterone

The concentration of corticosterone in plasma obtained from micro-hematocrit tubes was determined by radioimmunoassay. Briefly, 5 ul of either sample (5 ul in 495 ul buffer) or standard was incubated in a water bath at 80°C for 20 minutes and then cooled. To each tube was added 100 ul of the antibody to corticosterone and 100 ul ^{3}H-corticosterone. The tube was vortexed and incubated overnight in a refrigerator. The free ligand was separated by the addition of a charcoal suspension which was vortexed and incubated for 20 minutes at 4°C. The samples were centrifuged at 4,000 x g for 20 minutes. The supernatant was decanted into mini-scintillant vials. After the addition of scintillation fluid, the samples were counted for 5 minutes on a LKB beta counter. The complete assay appears in Appendix D.

ACTH

The concentration of ACTH in plasma was measured by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Briefly, 200 ul of either sample or standard and 100 ul of the Anti-ACTH were added to polypropylene tubes, vortexed and then incubated for 24 hours at 4°C. 100 ul of ^{125}I-ACTH was added to each tube, vortexed and incubated for an additional 24 hours at 4°C. The second antibody solution

(1.0 ml) was added to each tube, vortexed and incubated for 15 minutes at room temperature. The tubes were centrifuged at 1,500 x g for 30 minutes at 5°C. The supernatant was immediately aspirated. All tubes were counted on a Beckman 5500 gamma counter for 4 minutes. The lowest detectable amount is approximately 4 pg ACTH. The intra-assay variance for the Nichols Institute ACTH kit for 2 different pools was 6.1% and 9.7%. The inter-assay variance was 10.5% and 11.1%.

Sodium and potassium

Sodium and potassium concentrations in plasma were determined by flame photometry (Instrument Laboratories). Lithium was used as the internal standard. Samples (5 ul) obtained from micro-hematocrit tubes were analyzed in duplicate. Coefficient of variability for sodium was 2.4% intra-assay and 2.6% inter-assay, and for potassium 5.6% and 6.6% respectively.

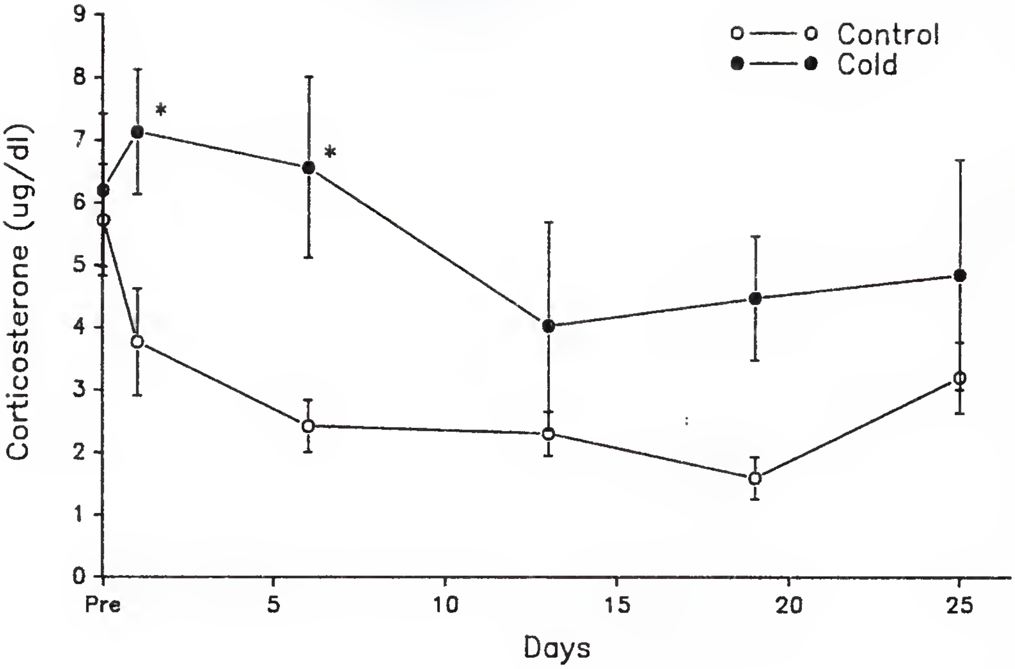
Statistics

Statistical analyses of data collected from cannulated rats were carried out by a two-way randomized block design, analysis of variance which tested for the main effects of environment and time. A posteriori pairwise comparisons were performed when appropriate, by a Duncan's New Multiple Range test which maintained the significance at the a priori level ($p<0.05$) (111). Changes in the concentrations of hormones collected at the time of sacrifice were determined by a one-tailed Student's t-test for independent samples, when appropriate. Significance was set at the 95% confidence level.

Results

Chronically cannulated rats.

The concentrations of certain vasoactive agents were measured in the plasma from rats sampled in their home environments under resting conditions. As expected, the concentration of corticosterone (B) in plasma was significantly increased by exposure to cold (Figure 5-1). The concentration of corticosterone remained significantly greater ($p<0.05$) than that of controls for nearly two weeks. There was no significant difference



Source	SS	DF	MS	F	P
Environment	18969.76	1	18969.76	30.74	<0.01
Time	10132.1	7	1447.44	2.35	<0.05
Env. * Time	3870.64	7	552.95	.896	
Error	62325.3	101	617.08		

Figure 5-1. The effect of exposure to cold on the concentration of corticosterone in plasma of chronically cannulated rats. Values are Mean ± S.E.M.
* Significantly greater ($p<0.05$) than control as determined by Duncan's New Multiple Range test.

between the mean values of the two groups when B was measured either after 13 or 19 days of exposure to cold, or after 25 days of exposure.

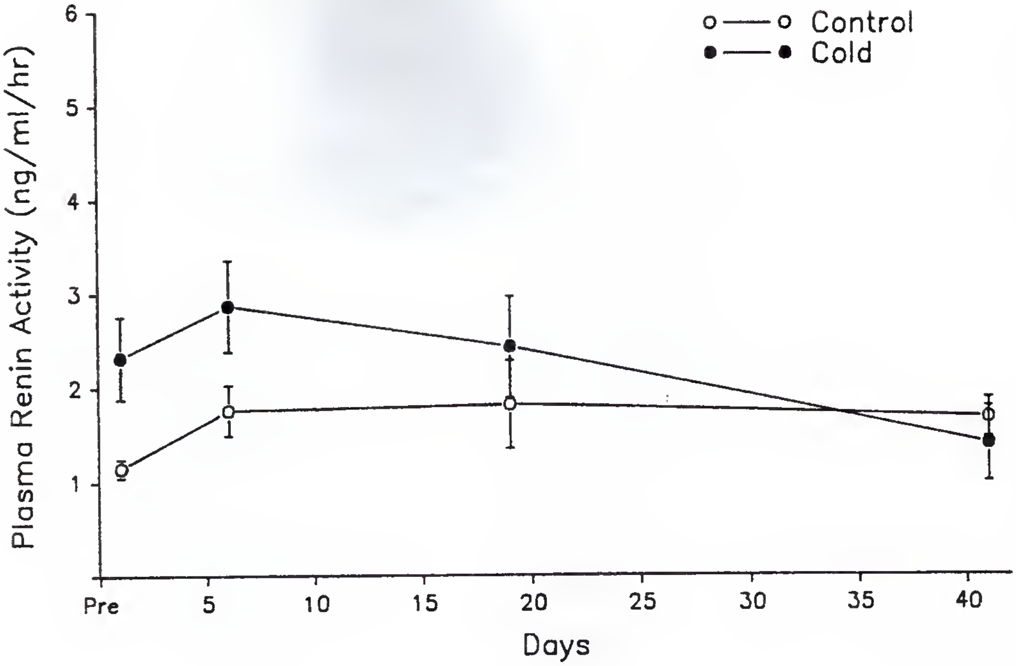
The effect of chronic exposure to cold on plasma renin activity (PRA) is shown in Figure 5-2. A significant main effect of environment ($p=0.048$) was found. The results suggest a transient or initial increase in PRA which was evident on the first, and seventh days of exposure to cold. No significant difference between groups was seen in PRA values on either day 19 or 41.

The effect of exposure to cold on the concentration of aldosterone in plasma can be seen in Figure 5-3. No significant main effect, either environment or time, nor a significant interaction ($p = 0.061$) was found. There was, however, a trend for the cold-treated group to reduce the concentration of aldosterone in their plasma. The concentration of aldosterone on the first day of exposure to cold differed significantly ($p<0.05$ by Duncan's Multiple Range test) from the concentration measured after 19 and 41 days of exposure to cold.

No significant effect of chronic exposure to cold was evident on either serum sodium or potassium concentration (Table 5-1). There was no significant difference between the control and cold-treated groups either prior to, or after 42 days of exposure to cold.

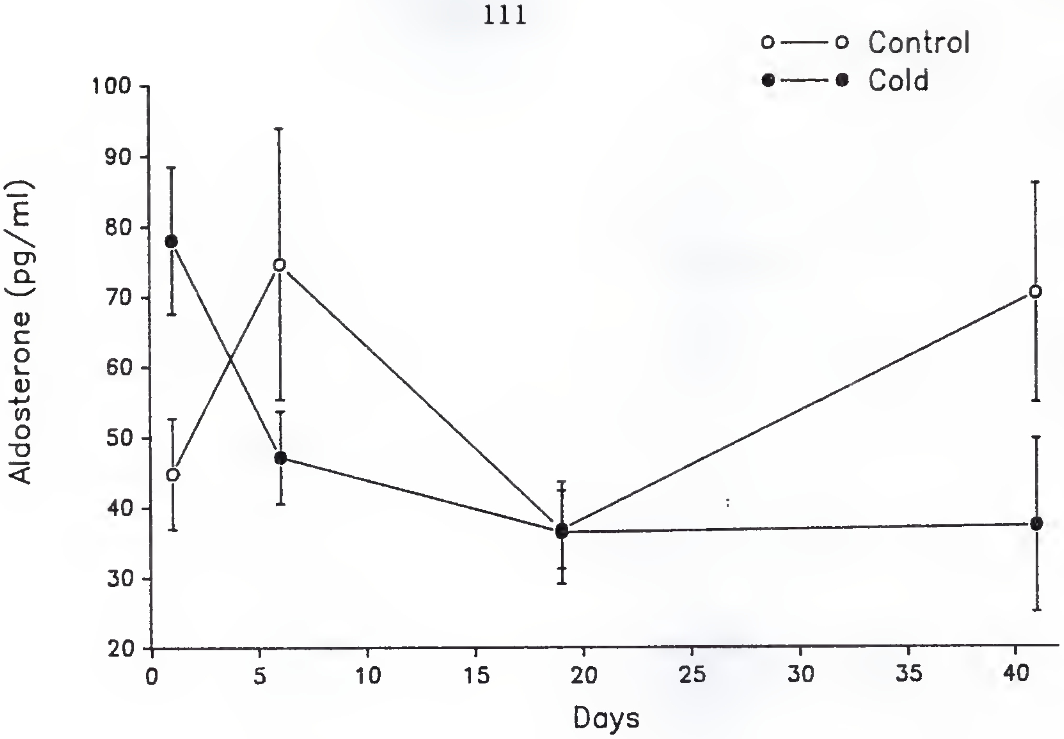
Chronic exposure to cold had no significant effect on the hematocrit (Figure 5-4). No significant main effect of cold, nor a significant cold x time interaction was found. However, there was a significant ($p<0.05$) main effect of time. Both the cold-treated and control groups had a significant decrease in hematocrit when day 1 was compared with pre-treatment values. In addition, after 42 days of exposure to cold, the cold-treated group demonstrated a significantly decreased hematocrit. This is a finding unique to this study and suggests the sampling of blood from these animals may have been excessive.

The relationship between certain vasoactive agents (PRA, B, and norepinephrine (NE)) and mean arterial blood pressure (MAP), diastolic blood pressure (DBP), and heart



Source	SS	DF	MS	F	P
Environment	4.443	1	4.443	3.999	<0.05
Time	4.147	3	1.382	1.244	0.304
Env. * Time	3.623	3	1.208	1.087	0.364
Error	51.116	46	1.111		

Figure 5-2. The effect of 42 days of exposure to cold on plasma renin activity of chronically cannulated rats.
Values are Mean ± S.E.M.



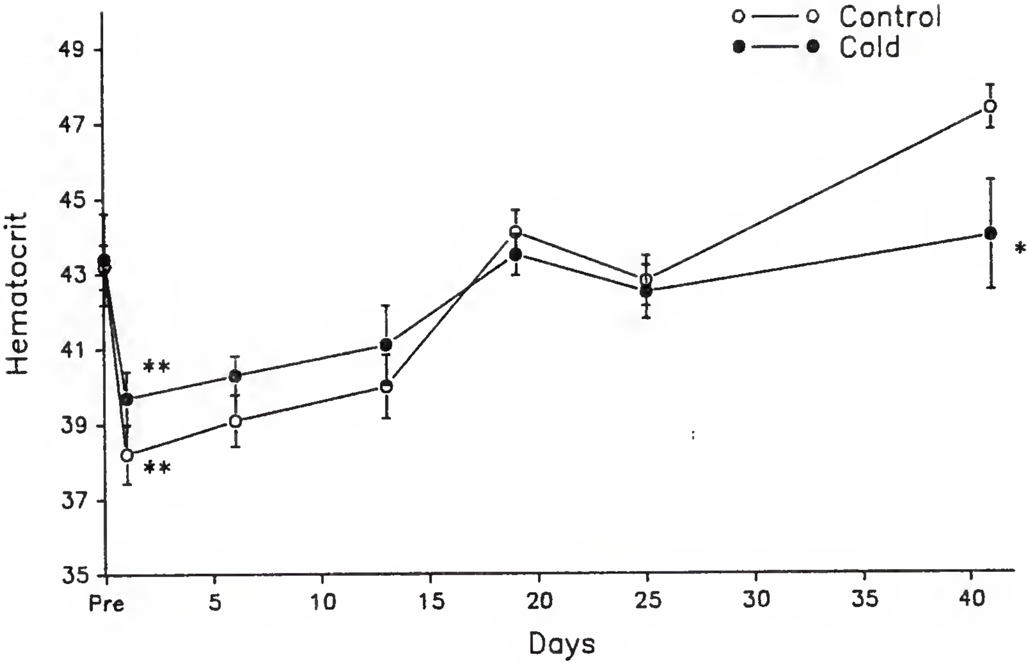
Source	SS	DF	MS	F	P
Environment	579.48	1	579.48	.532	
Time	4913.75	3	1637.92	1.50	
Env. * Time	8402.69	3	2800.9	2.57	
Error	51241.37	47	1090.24		

Figure 5-3. The effect of 42 days of exposure to cold on the concentration of aldosterone in plasma of chronically cannulated rats. Values are Mean ± S.E.M.
* Significantly less than (p<0.05) pre-treatment value as determined by Duncan's New Multiple Range test.

Table 5-1. The effect of 42 days of exposure to cold on the concentrations of sodium and potassium in serum of chronically cannulated rats.

	SODIUM (mEq/L)		POTASSIUM (mEq/L)	
	PRE	Day 42	PRE	Day 42
COLD	145.25 ± 2.58	136.50 ± 1.50	4.31 ± 0.41	4.80 ± 1.21
CONTROL	148.11 ± 3.39	148.33 ± 7.00	4.79 ± 0.50	5.60 ± 0.49

Values are mean ± S.E.M.



Source	SS	DF	MS	F	P
Environment	0.756	1	.756	.113	
Time	779.67	7	111.38	16.63	<0.01
Env. * Time	65.86	7	9.41	1.41	
Error	716.84	107	6.7		

Figure 5-4. The effect of 41 days of exposure to cold on the hematocrit of chronically cannulated rats.
Values are Mean ± S.E.M.
* Significantly less than ($p<0.05$) control as determined by Duncan's New Multiple Range test.
** Significantly less than ($p<0.05$) pre-treatment value of respective group as determined by Duncan's New Multiple Range test.

rate (HR) was determined by linear regression. The results of the regression analyses are reported in Table 5-2. In general, the correlations between the plasma concentration of the vasoactive agent and either blood pressure or heart rate were very poor ($r < 0.1$). The only relationship which exhibited a weak correlation was the concentration of NE versus HR ($r = 0.301$) of the cold-treated group.

Non-Cannulated Rats

The concentration of aldosterone, AVP, B, ACTH, sodium, and potassium, as well as osmolality, hematocrit and plasma renin activity were measured from trunk blood collected at the end of each experiment. The effect of exposure to cold on these variables from three separate experiments of varying duration, i.e. exposure to cold, is shown in Table 5-3. After twenty days of exposure, no significant differences were found between groups in their concentrations of either B or ACTH. Similarly, no significant difference was found in either the concentration of aldosterone, AVP or plasma renin activity in any of the experiments in which the duration of exposure to cold was greater than 20 days. The concentrations of sodium and potassium in serum as well as osmolality of the plasma were unaffected by exposure to cold. In general, the repeatability of the assays was quite good, as the data from the control group were very consistent. The concentrations of potassium in serum were slightly higher in animals (both cold-treated and control) when the samples were collected from trunk blood rather than directly from the arterial cannula. This is not an uncommon finding as collecting trunk blood may result in the mixing of gastric contents, which are high in potassium, with the blood sample.

The effect of chronic exposure to cold on the hematocrit is an interesting result. In each of the experiments in which the blood sample was collected from the trunk at the time of sacrifice, there is a significantly greater ($p<0.05$) hematocrit in the cold-treated group. This effect was found in two other experiments in which the duration of exposure to cold was also 20 days. Only in the one experiment in which the animals were chronically cannulated, and chronically sampled, was the hematocrit not increased

Table 5-2. The effect of chronic exposure to cold on the relationship between vasoactive agents and various hemodynamic parameters in the chronically cannulated rat.

$Y = B(1)X + B(0)$

		B(1)		B(0)		Correlation Coefficient	
X	Y	COLD	CONTROL	COLD	CONTROL	COLD	CONTROL
PRA:	MAP	-1.124	-2.350	118.20	101.649	0.033	0.041
	DBP	-2.109	-4.397	101.510	93.549	0.080	0.099
	HR	-1.269	3.223	457.100	346.039	0.003	0.005
B:	MAP	-0.096	0.058	118.965	96.151	0.080	0.019
	DBP	-0.111	0.016	103.322	85.004	0.151	0.001
	HR	-0.103	-0.074	429.083	356.978	0.006	0.002
NE:	MAP	0.003	-0.013	108.2	105.949	0.022	0.110
	DBP	-0.003	-0.008	97.219	91.889	0.028	0.036
	HR	0.041	0.003	386.268	358.611	0.301	0.001

PRA: Plasma Renin Activity (ng/ml·hr)
B: Plasma Corticosterone (pg/dl)
NE: Plasma Norepinephrine (pg/ml)
MAP: Mean Arterial Pressure (mm Hg)
DBP: Diastolic Blood Pressure (mm Hg)
HR: Heart Rate (bpm)

Table 5-3. The effect of exposure to cold on various vasoactive agents in rats.

No. of Days of Exposure	PRA (ng/ml/hr)		Aldosterone (pg/ml)		AVP (pg/ml)		B (ug/dl)		ACTH (pg/ml)	
	COLD	CONT.	COLD	CONT.	COLD	CONT.	COLD	CONT.	COLD	CONT.
20	2.3±0.4	2.7±0.3	71.0±15.7	44.6±9.3	3.9±1.0	3.2 ±0.1	7.2±2.0	3.6 ±2.9	21.7±0.8	31.0±8.4
35	2.6±0.4	2.7±0.4	137.5±42.2	152.5±34.4	5.0±0.4	4.2±0.8	nd	nd	nd	nd
42	1.4±0.4	1.7±0.2	37.3±12.3	70.3±15.6	nd	nd	nd	nd	nd	nd

No. of Days of Exposure	Hematocrit		Sodium (mEq/L)		Potassium (mEq/L)		Osmolality (mOsm/kg H_2O)	
	COLD	CONT.	COLD	CONT.	COLD	CONT.	COLD	CONT.
20	48.8±0.9*	46.8±0.4	138.5±0.6	140.1±0.3	8.5 ±0.2	8.0±0.1	283.0 ±1.9	284.1±1.3
35	50.5±0.8*	45.5±1.3	132.4±1.6	129.8±0.5	7.5±0.3	7.2±0.3	289.6±1.0	290.6±1.8
42	44.0±1.5*	47.4±0.6	136.5±1.5	148.3±7.0	4.8 ±1.2	5.6±0.5	nd	nd

Values are Mean ± S.E.M.
* Significantly different from control ($p<0.05$) by independent-sample, two-tailed t-test.

by exposure to cold. It became apparent, through following the hematocrit with each sample, that the animals in both groups were having difficulty replenishing their red blood cells between samples (Figure 5-4). For this reason, the animals were not sampled for 20 days (day 23 to day 42). The control group significantly increased their hematocrit, while the cold-treated group showed no such increase over the 20 days.

Discussion

The Role of Glucocorticoids in Cold-Induced Hypertension.

Acute exposure to cold is a potent stimulus to a large variety of hormones in humans and animals. Many of these hormones, such as ACTH, corticosterone, and epinephrine are readily stimulated in response to a variety of general stressors (restraint, fear, pain, exercise) in addition to cold. In the late 1930s, Selye (174) described a biologic stress syndrome which later became known as the General Adaptation Syndrome. The initial response to the noxious agent or stressor represents the somatic expression of the generalized call to arms of the body's defense systems (176). The initial response is typically characterized by a rapid increase in ACTH, corticosterone and epinephrine. This initial response is followed by the "stage of resistance", which represents an acquired adaptation as a result of either continued or repeated exposure to a stressor (176). The essence of the stressor, according to Selye (176), is the non-specific demand to adjust to an entirely new situation. The type of stressor, or stress producing agent, is immaterial in regard to whether the agent is pleasant or unpleasant. The intensity of demand for readjustment or adaptation is significant in the reestablishment of homeostasis (172). Clearly, exposure to cold represents a potent "non-specific stressor" as defined by Selye.

Since the time Selye formulated his hypothesis of a generalized "stimulus-independent" response, other investigators have also examined the nonspecificity of stress. When the concentration of corticosterone in plasma was used as the index of pituitary-adrenocortical activity, Mason (136) reported an increase in response to virtually any novel or unfamiliar stimulus. Similarly, Forberg et al. (52) reported an increased urinary output of catecholamines in response to viewing pleasant, fearful or comic films in humans. In contrast, a great deal of evidence supports an alternate hypothesis, in which either differential or specific responses are unique to each stimulus. Keim and Sigg (106) demonstrated that the temporal patterns of responses in hypothalamic content of norepinephrine and the concentration of corticosterone in plasma were different with respect to the evoking stimulus (restraint, footshock, or a novel environment). Similarly, Odio and Maickel (144) demonstrated unique or differential biochemical patterns of response to cold, immobilization, and footshock. These authors measured more than one variable, which they suggest results in a "finger-print" unique to each stimulus. The key to the differences seen recently, versus the initial work done by Selye, is two-fold: first, the differences or changes were examined over time; and second, multiple responses were measured. Therefore, temporal sampling is critical in describing the response to stress and in the reestablishment of homeostasis.

In the present experiment, chronically cannulated animals were exposed to low environmental temperature and a variety of hormones was measured throughout the duration of exposure. Acute exposure to cold resulted in an increase in the circulating concentrations of ACTH, corticosterone (B), and epinephrine (E). These results are not surprising. As mentioned above, these hormones respond to a variety of different stimuli. Increased concentrations of ACTH, B, and E in response to cold have been reported previously by many investigators (158,192). It is tempting to suggest, based on the similarities between exposure to cold and other stressors (immobilization, footshock and fear), that the cold-exposed rat is a chronically stressed model. Although the general trends (increased ACTH, B, E) are the same, the magnitude and duration of these changes are different. For instance, in response to exposure to cold, the concentrations of both NE and E in the plasma are increased, as they are with immobilization. However, at no time does the concentration of E in plasma in the cold-exposed rat exceed that of NE as it does with immobilization. Further, Popovic (156) reported an increased concentration of both ACTH and B in restraint-adapted animals, while the concentration of both ACTH and B are unchanged with adaptation to cold. It appears that the specific hormonal pattern of response which is stimulated by exposure to cold is different from other stressors. The general similarities may result from the physiological demands necessitated by exposure to cold (increased substrate, stimulation of skeletal muscle) which are similar to the "fight or flight" phenomenon. It appears immaterial with regard to whether the glucocorticoids are stimulated as part of either the generalized response to stress or in a specific pattern in defense of body temperature. What is important, is the role of each of these hormones in the survival of the animal.

During the initial exposure to cold, the major mechanisms for conserving body temperature are a decreased heat loss, and an increased heat production primarily by shivering. Stimulation of B, E, and glucagon each of which increase the concentration of glucose in the plasma is necessary, at least initially, to supply substrates for heat production. This is critical for survival in the cold. In the present studies, B and epinephrine, and presumably ACTH, were all increased in response to cold. This increase was transient, however, as none of these hormones was elevated after 20 days of exposure to cold (Figure 5-1, Table 5-3). Several conclusions can be drawn from these results. First, they suggest that provision of substrates during chronic exposure to cold is sustained without an increase in the concentration of these hormones in the plasma. This may result from a) a change in sensitivity to the hormones, either at the level of the receptor or beyond it; b) a change in utilization of substrate, possibly due to the switch from shivering to nonshivering thermogenesis which occurs after 7-10 days of exposure; or c) an increased availability of substrates, possibly due to the increased consumption of food which occurs after 7-10 days of exposure. Any or all of these could result in a decreased demand, and thus decreased circulating levels of the hormones which regulate availability of substrate. In addition, the clearance of these hormones from the blood may also be increased.

The possibility that the glucocorticoids or "general stress" hormones are involved in the development of hypertension is provocative. Whitworth et al. (198) infused either cortisol or deoxycorticosterone into healthy human volunteer subjects at levels which mimicked stimulation by ACTH in man. These authors reported a significantly increased systolic blood pressure which was maintained over the 5 day period of infusion (198). The idea of adrenocortical steroid-induced hypertension is not new. Various studies done in the early 1940s demonstrated the ability of both cortisone and deoxycorticosterone (DOC) to increase blood pressure (175). The use of DOC in combination with an increased salt intake is a familiar experimental model of experimental hypertension in rats. In addition to the original experiments using DOC and cortisone, a number of other steroids have been used to induce hypertension in several species of animals; for example: methylandrostenediol, corticosterone, 19-nor-progesterone, dexamethasone and ACTH. A description of the various steroids and animal models used has been reviewed by Scoggins et al. (171). These authors (171) present evidence for a "hypertensinogenic" activity of ACTH in sheep and humans, which is distinct from, or independent of, either its mineralocorticoid or glucocorticoid effects. Further, Scoggins et al. (171) hypothesize that the mechanism by which either ACTH or adrenocortical steroids induce hypertension is via a central "hypertensinogenic" receptor. This hypothetical receptor, when stimulated, results in an increased cardiac output and/or total peripheral resistance and thus, an elevation of blood pressure. It is interesting, that a rapid increase in both cardiac output and the concentration of ACTH in plasma are prominent responses to acute exposure to cold. As discussed above, the generalized response to stress involves not only an increased rate of secretion of ACTH, but an increased rate of release of catecholamines as well. Interestingly, chronic infusion of ACTH does not induce hypertension in dogs (129) unless the concentration of NE in the plasma is increased (127). Impairment of renal excretory capability potentiates the hypertensive effect of ACTH, for example reduction of renal mass (198), or co-administration of angiotensin II (129). This suggests that ACTH induced-hypertension is renal-mediated. Woods et al. (200) provide strong evidence for a renal mechanism in ACTH/NE-induced hypertension in dogs. When ACTH was infused while the concentration of NE in plasma was elevated, a moderate hypertension and natriuresis were clearly evident. However, when the authors servocontrolled renal perfusion pressure at normal levels, infusion of ACTH resulted in a marked sodium retention and severe hypertension. This suggests that pressure-induced natriuresis actually blunted the chronic hypertension associated with the sodium-retaining effects of ACTH (200). Further, these authors (200) concluded that ACTH induces hypertension by a reduction in renal excretory capability which alters the setpoint of the curve characterizing renal function (arterial pressure-natriuresis).

Chronic exposure to cold results in several similarities with ACTH/NE-induced hypertension. In addition to the increased concentrations of ACTH and NE, there is an increased cardiac output, a large diuresis and the maintenance of sodium balance. Whether exposure to cold results in an alteration of renal excretory capability, and therefore a resetting of the curve characterizing renal function, is undetermined. Further, it is unknown whether a transient elevation of ACTH, similar to that seen with exposure to cold, is sufficient to induce alterations in renal function as suggested by Woods et al. (200). The possibility of a interaction between ACTH and NE in cold-induced hypertension requires further study, but may suggest a mechanism by which stress potentiates hypertension and cardiovascular disease in humans as well.

Renin-Angiotensin II-Aldosterone System in Cold-Induced Hypertension.

The secretion of renin, and thus, the formation of angiotensin II (AII), is primarily controlled by the sympathetic nervous system (SNS). The stimulation of beta-adrenergic receptors results in an increase in output of renin by the kidney. In addition to stimulation by circulating catecholamines, renin is released in response to direct stimulation of the renal nerve (126). The enzyme, renin, acts on angiotensinogen to form angiotensin I which is converted to the octapeptide, AII, by angiotensin I converting enzyme. Angiotensin II is one of the most potent vasoconstrictor agents produced physiologically.

A great number of experimental studies and theoretical analyses suggests that the kidneys play a key role in the long-term regulation of blood pressure (75,79). The relationship between arterial pressure and renal excretion of water and sodium, or the pressure-induced diuresis-natriuresis is central in this hypothesis. Basically, the idea is that the ratio between renal excretory capability and the intake of water and electrolytes is reduced. This occurs primarily as the result of a decreased renal excretory capability. Upon first examination of this hypothesis, it would seem that it would only be applicable in models which involve a fluid expansion, as a decreased renal excretory capability should alter sodium and fluid balance, increase blood volume, and thus result in hypertension. According to Guyton (76), this need not be the case. An initial, but transient, increase in blood volume may occur, but during sustained hypertension, no increase in either blood volume or extracellular fluid will be detectable by typical methods. A shift in the renal function curve (RFC) which relates arterial pressure to sodium output/intake to the right of its normal position will require arterial pressure to rise in order to achieve sodium balance. The arterial pressure will continue to rise until the line of the curve of renal output intersects with that of sodium intake, and thus, the reestablishment of sodium balance. Different factors are known to alter the RFC and can be divided into either intrinsic or extrinsic factors. Some of the intrinsic factors which can alter the RFC and which may be altered during exposure to cold include: constriction of renal arteries, either afferent or efferent arterioles, and changes in tubular reabsorption. Extrinsic factors include: changes in the concentrations of aldosterone, vasopressin, and renin-AII, as well as changes in nervous stimulation to the kidney, or concentrations of electrolytes in plasma. Each of these factors has received an extensive review (75). The renal hypothesis has received extensive support by numerous investigators and has recently been summarized (78). Recently, one very important paper has been published which demonstrated a rightward shift of the renal function curve in patients with secondary forms of hypertension. This study by Kimura et al. (108) is the first to utilize renal function curves in humans to study the pathogenesis of hypertension and the ways in which therapy can return hypertensive patients to normal.

Exposure to cold induces hypertensin in rats and is characterized by an increased activity of the SNS. Since plasma renin activity is controlled by the SNS, it is logical to assume that exposure to cold would be accompanied by an increased concentration of AII in the plasma. An increased concentration of AII could act directly at the vasculature to produce vasoconstriction and thus increase blood pressure. Plasma renin activity (PRA) is often used as a measure of AII. The present study used chronically cannulated cold-treated and control rats, to determine whether an increased output of the SNS resulted in an increased PRA. The effect of chronic exposure to cold resulted in a significant main effect of cold (p=0.48) on PRA (Figure 5-2). However, post hoc analysis using a Duncan New Multiple Range test failed to find a significant increase in PRA at any particular time during exposure to cold. This is interesting for several reasons. First, it emphasizes the need for repetitive sampling within the same animal and the need to follow such a variable over time. Second, it suggests that the changes in PRA and AII which occur in response to exposure to cold are small, subtle changes, not dramatic alterations from control. Increased PRA was seen through the first week, and was absent when measured after 19 days of exposure to cold. Further, PRA was not significantly increased when measured at any time after 19 days of exposure to cold (Table 5-3). It is interesting that the increase in PRA disappears during the time typically associated with the greatest increase in blood pressure (day 13). This return of PRA to control levels also occurs at a time when the activity of the SNS remains elevated. Similarly, the concentration of aldosterone tended to be high on the first day of exposure to cold, but declined as exposure to cold continued. The results of the present studies are similar to those of others who reported either no change (93,199) to cold-pressor stimulation or a slight decrease (199) to acute cold in humans. Similarly, Katovich et al. (105) reported no difference in PRA of rats exposed to cold for 6 weeks. There are several possible explanations for the lack of a sustained increase in PRA. First, renal sympathetic nerve activity may decrease reflexly in response to the increased blood pressure. Second, it may be attributed to the decreased circulating level of epinephrine. Regardless of the mechanisms which are involved in returning PRA to control levels, the most relevant question is whether the transient elevation of PRA could have contributed to cold-induced hypertension.

Exposure to cold resulted in an increased activity of the renin-AII system. Further, the circulating levels of catecholamines, and thus the activity of the SNS was also shown to be increased (Chapter Four). In response to chronic exposure to cold, food intake, and thus electrolyte intake, is significantly increased (Chapter Two). In addition, the concentration of corticosterone is also increased transiently (Chapter Five). Any one of these alterations alone, or perhaps in combination with one another. may play a major role in altering renal function. Whether the renal function curve is displaced to the right in cold-induced hypertension as it is in DOCA/salt (181), SHRs (143), aldosterone-infused or volume loading hypertension (75), AII infused (40) and Goldblatt renal hypertension (142) remain to be investigated.

In the present studies, PRA was elevated during the first week of exposure to cold. Presumably, PRA remained elevated through the first 19 days of exposure, as did the concentration of AII. This is an hypothesis, as the data from the intermittent point was lost due to a methodological error. A possibility exists that AII may have raised blood pressure by acute vasoconstriction. This seems unlikely, as the concentration of AII in the plasma would not be expected to be high enough. Possibly, AII increased blood pressure via its slow pressor effect as proposed by Cowley and McCaa (34). The hypothesis of a slow pressor mechanism for AII was examined by Bean et al. (9). Basically, sub-pressor doses of AII were infused into conscious dogs over a two week period. The acute pressor effect of AII was then measured by standard dose-response techniques. These authors (9) reported a gradual increase in mean arterial pressure during the period of infusion. Most importantly, prolonged infusion of AII shifted the dose-response curve upward, i.e. a greater blood pressure resulted at any given dose of AII administered acutely (9). Thus, previously sub-pressor doses of AII resulted in an increased blood pressure. With regard to mechanisms of the slow-developing pressor response, Lohmeier and Cowley (128) studied the effects of chronic intrarenal infusion of AII. These authors (128) reported a pronounced antinatriuresis (and antikaliuresis) which immediately followed intrarenal infusion, but persisted only for the first 24 hours. After 24 hours, sodium and fluid balance were achieved. The sodium retaining property of AII was capable of overriding the pressure-natriuresis which would normally be expected. The authors suggest that AII may act via a potentiation of the vasoconstrictor response to adrenergic stimulation in the vascular bed of the kidney (128). Whether AII results in a change in renal function during the initial exposure to cold, which is then maintained by the alteration of the AII dose-response curve, is unknown. It is intriguing however, that with chronic exposure to cold, the PRA was increased to sub-pressor levels, and the circulating concentration of NE was elevated. The combined effects of an increased concentration of NE in the presence of an altered AII dose-response curve in the cold remains to be determined.

In addition to the possible mechanism discussed above, angiotensin II may contribute to the sustained elevation of blood pressure by central mechanisms. The central effects of AII with regard to provoking drinking are well known (62). In addition, injection of AII at the subfornical organ (35), at the area postrema (101, and at the superficial structures of the dorsomedial medulla (26), results in an increased blood pressure. Recently, Andreatta et al. (3) have demonstrated a dose-dependent relationship between AII applied topically to the rostral ventrolateral medulla (RVLM) and increased blood pressure. As previously discussed, it is the RVLM region which is hypothesized by Reis et al. (164) as the region which is involved in the regulation of the output of the SNS. Interestingly, several authors (28,169) have shown high levels of angiotensin converting enzyme colocalized with the catecholamine-secreting types of cells within the ventrolateral medulla. These results raise the interesting possibility for AII to modulate sympathetic outflow centrally; specifically at the level of the RVLM.

In addition to modulating outflow of the SNS, sufficient evidence has been presented to suggest that central AII may modulate baroreceptor reflex control of heart rate. Direct microinjection of AII into the nucleus tractus solitarius (NTS), the region of baroreceptor reflex control, results in dose-dependent changes of mean arterial pressure and heart rate (26). In addition, Casto and Phillips (27) demonstrated an attenuation of the baroreceptor reflex control of heart rate with infusion of AII into the NTS. Similarly, microinjection of a specific AII antagonist into the NTS resulted in a selective facilitation (to increased but not decreased pressure) of the baroreceptor reflex control of heart rate in response to increased mean arterial pressure (22). In the cold-exposed rat, the sensitivity of the baroreceptor reflex to an increased arterial pressure was significantly depressed. The altered baroreceptor reflex results in a disinhibition of the activity of the SNS, thus altering blood pressure.

In the present studies, the elevated PRA may have contributed to the increased blood pressure by several mechanisms including a) a direct vasoconstrictor affect; b) decreased sensitivity of the baroreceptor reflex; c) potentiation of the renal vasoconstrictor responses to NE; d) central stimulation of the area postrema, subfornical organ or dorsomedial medulla; and e) central stimulation of the RVLM to increase sympathetic stimulation. In the present studies, alterations in the central renin-angiotensin II system were not determined. However, Papanek and Fregly (152) have demonstrated an increased dipsogenic responsiveness to centrally administered AII. This strongly suggests that chronic exposure to cold results in changes within the central renin-angiotensin system. The extent and significance of such changes remains to be investigated. Further, if the initial increase in AII was sufficient to shift the AII dose-response curve as shown by Bean et al. (9), then the return to basal concentration of AII may have also played a role in the maintenance of the elevated blood pressure.

Changes in Arginine Vasopressin During Chronic Exposure to Cold.

Arginine vasopressin (AVP) is a multifunctional hormone. The most familiar physiological effect of AVP is its antidiuretic properties which act in the kidney. Maximal antidiuretic activity occurs in the range of 10-20 pg/ml. In addition, AVP is a very potent vasoconstrictor agent in physiological concentration. Often, the role of AVP on the circulatory system is overlooked, as the pressor activity of AVP is not clearly apparent until concentrations in the blood exceed 30 pg/ml. The vasoconstrictor activity of AVP is much greater however, with significant increases in total peripheral resistance of 10-20% occurring with concentrations of 5-20 pg/ml in plasma, i.e. the same range as its antidiuretic properties (31). Chronic exposure to cold results in hypertension and an hypothesized volume contraction. These two situations influence AVP is opposing directions. Thus, it was difficult to predict how the concentration of AVP in plasma would change. It was hypothesized that volume-contraction would stimulate AVP, and an increased concentration of AVP may contribute to the elevation of blood pressure seen during chronic exposure to cold.

The concentration of AVP in the plasma was measured in hypertensive rats which had been exposed to cold for 21 days. No difference in the concentration of AVP was found (Table 5-3). This suggests that AVP does not contribute to the sustained elevation of blood pressure. In addition Lenquist (123) reported a decreased concentration of AVP in response to an acute exposure of man to cold. Cold-induced diuresis may result from either an inhibition of, or a decreased sensitivity to, circulating AVP (58). Previous work from this laboratory (6) reported a decreased responsiveness of rats to acute administration of pitressin, as well as an inability of cold-treated animals to concentrate their urine in response to a 24 hour dehydration. Thus, an increased concentration of AVP is unlikely to occur during acute exposure to cold. During chronic exposure to cold, the increased activity of the SNS results in vasoconstriction, a decrease in blood flow to the periphery, and a decreased venous compliance. This should result in an inward shunting of blood, and an increased central venous pressure. An increased central venous pressure may stimulate the diuresis seen with exposure to cold, and should likewise inhibit the release of AVP. Even after a diuresis and a sustained volume contraction, central venous pressure could be maintained by the inward shunting of blood. Thus, there should be no stimulus to increase AVP. If this is the case, the rat would regulate extracellular fluid at a perceived normal volume, although the actual total volume is reduced. Whether chronic exposure to cold results in a decreased total volume is undetermined, but would provide an interesting mechanism invoked by the animal to conserve body temperature. It addition, it would provide an explanation for volume-contraction in a hypertensive model.

Summary

Chronic exposure to cold represents a unique method of induction of hypertension in rats. A number of transient hormonal responses occur, each of which may contribute to the elevation of blood pressure. Exactly which of these vasoactive agents initiates the hypertension, and by which mechanisms remains to be investigated. It seems reasonable, that cold-induced hypertension is not the result of one discrete alteration, but is as multifactorial and complicated as essential hypertension. Further, chronic exposure to cold has several similarities with the general stressful responses present in humans, particularly with regard to hormonal changes. Chronic exposure to cold may provide an opportunity to examine the role of such stress-induced hormones, within their normal physiological concentrations, in the control of the circulatory system. Further, this model provides the unique opportunity to investigate the effect of increased activity of the SNS and the interaction between elevated levels of NE and a variety of vasoactive agents.

CHAPTER SIX
SUMMARY

Cardiovascular diseases represent a major form of mortality and contribute in a great degree to the morbidity in the United States. According to epidemiological and clinical reports, most notably the Framingham Study (103), lethal cardiovascular diseases (coronary, stroke, and congestive heart failure) account for more than half of the annual death toll. One of the most common and most potent precursors to the cardiovascular diseases is a sustained increase in arterial pressure. Because of its significant role in cardiovascular diseases, the hypertensive syndrome has been the emphasis of extensive research. A large number of experimental models, each of which shares one or many of the features seen in human essential hypertension, has been developed. These experimental models were briefly reviewed in Chapter one. The need to develop new models that may contribute additional information to an understanding of the syndrome of hypertension is clear.

The use of animal models is important for investigating the initiating factors and pathogenesis, as well as for developing treatments and therapy for human diseases. According to Gross (71), a good hypertensive model should be a) simple to use; b) uniformly reproducible; c) relatively inexpensive; d) feasible in small animals; e) use minimal quantities of compounds; f) able to predict therapeutic antihypertensive properties.

Acute and chronic exposure to low environmental temperature is a naturally occurring phenomenon. As a result, the body is naturally well equipped with mechanisms designed to ensure survival in response to the environmental challenge. Exposure to cold results in a variety of physiological responses elicited in defence of internal or core temperature. One such response, which develops during chronic exposure to cold, is a sustained increase in arterial blood pressure. This sustained elevation of blood pressure has received relatively little attention. The present studies have focused on the question of whether chronic exposure to cold results in the development of hypertension as originally suggested by Fregly et al. (61). Further, studies were designed to investigated potential mechanisms which may have contributed to the development of hypertension.

The first series of studies was designed to test whether chronic exposure of rats to low environmental temperature (6°C or 41°F) induced a) a sustained elevation of blood pressure; and b) cardiac hypertrophy, a characteristic feature of hypertension. When male, Sprague-Dawley rats were continuously exposed to cold for 50 days, systolic blood pressure was significantly ($p<0.05$) increased (Chapter Two). The first significant increase in systolic blood pressure occurred after 21 days of exposure to cold (Chapter 2). In addition, the weight of the whole heart, left and right ventricle, as well as the atria was significantly ($p<0.05$) increased in the cold-treated group.

The results from the first study are important because they suggest that chronic exposure to cold induced hypertension in rats. Chronic exposure to cold represents a unique method for the induction of hypertension. In evaluating this model in accord with the criteria suggested by Gross (71) for experimental models of hypertension, the present studies demonstrated that exposure to cold is a) simple and relatively inexpensive; b) readily performed in small animals; c) uniformly reproducible (Chapters Two-Five); and d) does not require the administration of pharmacological quantities of drugs, i.e. uses no drugs. In addition, the advantages of chronic exposure to cold as a model include a) no surgical intervention; b) a naturally occurring physiological response; and c) a strong central and peripheral neurogenic component. A reproducible neurogenic model of hypertension has been elusive. In a naturally occurring model of hypertension, as with any model, the ultimate question which must be answered concerns mechanisms of induction or etiology.

In the first series of studies, chronic exposure to cold also affected the rate of gain in body weight, as indicated by a significant ($p<0.05$) interaction between environment and time (Chapter Two). The slope of the line relating body weight to time was depressed in the cold-treated animals. This occurs in the presence of a markedly stimulated food intake; the cold-treated animals consumed 60% more food on a daily basis. The increased intake of food, as well as the depressed body weight, is most likely the result of the hypermetabolic or thermogenic state of the animal. Likewise, these animals had a decrease in their white fat content, and an increase in the weight of their interscapular brown adipose tissue. These are characteristic changes associated with acclimation to cold. Because the animal was limited in the selection of food, the increased intake was accompanied by an increased intake (60% greater than controls) of sodium. Thus, in order to meet the increased caloric demand associated with exposure to cold, the animal increased its consumption of food, and by necessity, increased its intake of sodium. This raised the interesting question of whether cold-induced hypertension could be attributed to the increased intake of sodium in the diet.

Support for a role of sodium in hypertension stems primarily from the effective use of sodium restriction and/or administration of a diuretic as a major antihypertensive therapy, and from epidemiological and clinical studies which suggest a relationship between the intake of sodium and the incidence of hypertensive disease. Therefore, a study was designed to investigate whether the increased intake of sodium associated with chronic exposure to cold contributed to cold-induced hypertension. On the first experimental day (first day of exposure to cold), the cold-treated group was switched from a diet which contained 0.3% sodium (same concentration as that previously used), to a low sodium diet (0.15%). Both diets were specially formulated Hartroft sodium test diets (United States Biochemical Corp.). Since the cold-treated rats eat approximately twice as much food as controls, the intake of sodium would be approximately equal. The development of cold-induced hypertension was not altered by a reduced sodium diet (Chapter Three). Both the time-course, or the amount of exposure necessary for an elevation of pressure, and the magnitude were unchanged. In addition, the significant hypertrophy of the whole heart, right and left ventricles was unaffected by a reduced sodium intake. The results from this study failed to indicate an association between the increased sodium intake which occurs during chronic exposure to cold and the induction of hypertension. These studies were designed to investigate the effect of an increased sodium consumption on blood pressure, and as such, do not eliminate the possibility of an indirect effect of sodium in this or other models of hypertension, since the intracellular sodium content, sodium transport changes, and other sodium dependent processes were not investigated.

Survival at low environmental temperatures is dependent on the ability of the animal to reestablish a state of heat balance; i.e. where heat loss equals heat production. A reduction in heat loss is achieved primarily by increasing peripheral vasoconstriction and decreasing blood flow, thereby reducing the thermal gradient from the skin to the air, and by piloerection. An increase in heat production is accomplished by a variety of mechanisms including the following: shivering, direct heat producing effects of thyroxine and epinephrine, increased muscular activity, and non-shivering thermogenesis. The majority of these heat loss/production processes are mediated by an increased output from the sympathetic nervous system (SNS). Indeed, a characteristic feature of adaptation to low environmental temperature in rats is a large increase in the activity of the SNS. It is also well known that the SNS controls both minute-to-minute and long term arterial pressure. In addition, there is a great deal of evidence which suggests that alterations occur within the SNS in many forms of experimental hypertension (202). The question of whether the SNS is involved in the development of cold-induced hypertension stems logically from the role of the SNS in the regulation of both arterial blood pressure and internal temperature.

In the present studies, a profile of the SNS in the chronically cannulated rat exposed to low environmental temperature was described (Chapter Four). The SNS was described with regard to the concentration of the neurotransmitter, norepinephrine, in plasma, the concentration of the adrenal medullary hormone, epinephrine, and the activity of the baroreceptor reflex. These studies are unique, as they are the first to use chronically cannulated rats to measure directly blood pressure, and the resting concentrations of hormones in plasma of cold-treated animals, while in their home environments. Several important findings resulted from these studies with regard to the elevation of blood pressure and the activity of the SNS. First, acute and chronic exposure to cold resulted in a large sustained increase in the activity of the SNS as indicated by the increased concentration of NE in both plasma and urine (Chapters Two and Four). The increase in concentration of NE in the plasma was apparent within 24 hours of exposure to cold. Further, the concentration of NE in the plasma did not return to control levels even after 42 days of exposure. It is tempting to suggest that the excess activity of the SNS is directly responsible for the vasoconstriction, and thus, the sustained increase in arterial pressure. However, it was shown that the concentration of NE increased immediately, while the elevation of blood pressure (mean arterial pressure) did not occur for nearly two weeks (Chapter Four). This may suggest that compensatory mechanisms were evoked to normalize an initial elevation of blood pressure. It is reasonable to assume that the output of the SNS is not uniform; i.e. the degree of stimulation of the vasculature to the skin by the SNS was greater, and therefore preferentially vasoconstricted as compared with the degree of constriction of the vessels to the internal viscera, for example. If the increased resistance was not compensated for with a decreased vasoconstriction, and thus decreased resistance elsewhere, blood pressure would rise, since mean arterial pressure (MAP) is a function of cardiac output (CO) X total peripheral resistance (TPR). A likely candidate for vasodilatation initially is the skeletal muscle; one site which could greatly contribute to heat production. Perhaps, as heat production is shifted from mechanical to biochemical mechanisms, skeletal muscle vasodilatation is decreased, and thus TPR is increased. The redistribution of blood flow during exposure to cold remains to be investigated, but could provide a possible mechanism whereby SNS-induced vasoconstriction alone could sustain an elevation of blood pressure in the later stages of this model.

Another important finding in these studies resulted from the comparison of two different techniques used for the measurement of systolic blood pressure (SBP). In two different studies, exposure to cold for 42 days increased SBP to 153 ± 4 mm Hg whether measured indirectly using the tail-cuff technique or directly from an indwelling cannula. However, a particular advantage was afforded with regard to sensitivity by using indwelling cannula or direct assessment. In the first series of studies (Chapter Two) which indirectly measured SBP from the tail, the first significant ($p<0.05$) increase was seen after 21 days of exposure to cold. In the later series of studies which measured SBP directly, the first significant increase was seen on day 4, that is, the first day these measurements were made. The difference between the two studies is most likely the result of the use of restrained versus resting control animals, as the SBP of control animals was consistently higher when measured by the tail-cuff technique. Further, both groups (cold and control) displayed greater intra-group variability when pressures was measured by this technique. In summary then, both techniques provided similar results with regard to the final outcome of the study, i.e. the magnitude of the elevation of blood pressure. However, direct measurement from an indwelling cannula is required when the detection of small changes, or specific time-course of initiation of changes, is desired.

A direct effect of the SNS on the vasculature is but one mechanism by which the SNS may contribute to cold-induced hypertension. An additional mechanism whereby excessive output by the SNS may indirectly contribute to increased vascular resistance is by the sustained elevation of cardiac output. Cardiac output is significantly increased by exposure to cold (10,88,89). Increased cardiac output without a decrease in TPR will in itself increase blood pressure ($MAP = CO \times TPR$). However, like the increased activity of the SNS, the increased CO in response to cold-exposure, is an immediate response, and as such, is unlikely to contribute directly to the hypertension.

That a sustained increase in cardiac output may indirectly result in an elevation of blood pressure has been suggested by Folkow (50). Folkow has developed the concept of vascular structural reinforcement (50). Basically, an increased CO will stretch or distend arterioles. In response to distention by the higher pressure, the smooth muscle cells of the arterioles will hypertrophy. Hypertrophy of vascular smooth muscle cells is accompanied by a narrowing of the lumen of the vessel, and hence, an increased resistance to flow (500. In addition, Folkow (50) suggested that hypertrophied vessels with an increased wall/lumen ratio overrespond to vasoconstrictive stimuli. The work of Fregly et al. (60) and Bryar et al. (19) as well as by this author (unpublished data) have shown a decreased alpha-adrenergic or vasoconstrictor response in vitro in isolated vascular smooth muscle from animals chronically exposed to cold. These results do not support a role for a cardiac output-induced alteration of vascular resistance. However, further studies, which include histological assessment of cold-induced changes within the vascular smooth muscle during the development of cold-induced hypertension, will be needed to resolve this question.

An increase in the activity of the SNS stimulates the release of the enzyme, renin, from the kidney. Renin converts angiotensinogen to angiotensin II (AII). Therefore, the question of whether chronic exposure to cold results in an increased concentration of AII, as assessed by plasma renin activity, was investigated.

In the present studies, a transient, but significantly increased, plasma renin activity (PRA) was demonstrated in response to cold (Chapter Five). The potential for the renin-angiotensin system to influence the control of blood pressure was discussed in Chapter Five. Briefly, it is well known that angiotensin II (AII) influences blood pressure and interacts in a variety of ways with the SNS. For instance, peripheral AII has been shown to a) stimulate transmitter biosynthesis (15); b) inhibit transmitter reuptake (150,154); c) stimulate transmitter release (11,130), and d) stimulate the adrenal medulla (155,165) and sympathetic ganglia (187). In addition, the effects of central administration of AII on blood pressure are also well documented. These effects may be both direct and indirect;

for example, stimulation of the area postrema by AII influences the vasomotor centers, and thus output of the sympathetic and parasympathetic nervous systems (101). In addition, Sumners et al. (182) demonstrated a facilitation of the release of NE by AII in cells cultured from brain, which may suggest that AII modulates noradrenergic neural transmission centrally as well as peripherally. Whether the transient increase in AII seen in the present studies during chronic exposure to low environmental temperature contributed to the cold-induced hypertension, and, if so, by which mechanisms is unclear. Further studies in which the physiological effects of central and/or peripheral AII are blocked (either captopril or specific antagonists) are required to answer specifically whether AII plays an important role in either the development or maintenance of cold-induced hypertension.

The control of blood pressure is both complex and multifactorial. Basically, arterial pressure is the result of cardiac output and total peripheral resistance, as described by the equation MAP = CO X TPR. Cardiac output is a function of total blood volume, heart rate, stroke volume, resistance to venous return and vascular capacitance or compliance (76,77,80). Each of these components is influenced by autoregulatory mechanisms and circulating vasoactive agents, as well as being controlled by the autonomic nervous system. Total peripheral resistance is similarly under the control of the autonomic nervous system and influenced by vasoactive hormones such as angiotensin, bradykinin, vasopressin, serotonin, prostaglandins, and catecholamines. An alteration in any one or more of these factors can result in the development of hypertension. In addition, the same alteration in a regulatory mechanism may occur for a variety of reasons. For example, a change in renal function which affects blood volume, and thus cardiac output, may result from excessive sympathetic stimulation, alterations in the renin-angiotensin system, or from other extrinsic and intrinsic factors. Although the primary defect or etiology may be unknown, the ultimate response would be unifying, i.e. a change in renal function and thus hypertension.

The temperature of the body is regulated, like many physiological systems, via a feedback loop. Temperature sensors located both peripherally and centrally, provide information about body temperature to the central temperature regulatory centers. It is generally accepted that the centers involved in the regulation of body temperature are located within the hypothalamus. Specifically, the hypothalamic area which responds to cold is in the posterior hypothalamus approximately at the level of the mammillary bodies (21). Located within the posterior hypothalamus are sympathetic centers which affect vasoconstriction. An inhibition of this region results in intense vasodilatation. In contrast, stimulation of this sympathetic center results in vasoconstriction of the skin throughout the body. In addition, the dorsomedial portion of the posterior hypothalamus located near the wall of the third ventricle, is a center responsible for controlling shivering. Normally, this region is inhibited by signals indicating normal temperature, and is stimulated by signals indicating cold. In addition, E released primarily from the adrenal medulla and NE released primarily from sympathetic nerve terminals stimulate chemical thermogenesis, i.e. nonshivering thermogenesis (NSTG). The degree of NSTG that occurs is proportional to the amount of brown fat which exists, as brown fat contains vast amounts of mitochondria and is richly innervated by the SNS. With acclimatization to cold, both the intensity and capacity of NSTG is increased. In addition, in the case of the rat, the proportion of brown fat is increased. In addition to the effects of catecholamines on NSTG, thyroid, as well as glucocorticoid hormones, are important to survival at low environmental temperatures and have been the subject of intense review (57,59). Acute exposure to cold is a potent stimulus to increase ACTH, glucocorticoid hormones (cortisol or corticosterone), and epinephrine.

It is interesting to hypothesize that the central temperature regulatory centers located in the hypothalamus either influence or override the blood pressure control centers in the brainstem. The possibility of a hierarchy of control is supported by a similar example of exercise, in which higher centers of the brain appear to override the reflex control of blood pressure and heart rate to maintain the desired pressure. This may potentially occur at a variety of levels, and it is interesting that many of the variables elicited in defense of internal temperature are in some way linked to control of blood pressure. Exposure to cold alters food and water intake, urinary output, activity of the SNS, and the renin-angiotensin system. Each of these may affect the control of blood pressure directly, or by interacting with various other regulatory systems.

It appears that in response to low environmental temperature, the rat has evoked regulatory mechanisms whose primary function is the defence of body temperature and which therefore guarantee survival. A cost or side-effect of these alterations is the development of hypertension. Chronic exposure to cold is thus a unique, naturally occurring model for inducing hypertension which does not involve either surgical manipulation, or the administration of pharmacological dosages of drugs.

It does not appear that an environmental effect on blood pressure is unique to the rat or laboratory animal. There is strong evidence to suggest that this form of hypertension exists in a variety of other experimental animals, as well as in humans. An increased blood pressure in response to acute exposure to cold has been demonstrated in sheep (185) and in young oxen (10). In 1950, Gilson (6) reported an elevation of systemic blood pressure in rats which were adapted to cold. Later, Fregly (5) as well as Heroux and Dugal (87) reported a sustained increase in systolic blood pressure in cold-exposed rats. A significant negative correlation of ambient temperature on blood pressures in humans has been demonstrated in a variety of longitudinal studies conducted in Great Britain and United States (Chapter One). In addition, Reed et al. (162) have reported a significant elevation of mean arterial pressure in young men stationed for 42 weeks in Antarctica. Mean arterial pressure increased 16 mm Hg to 101 ± 3 mm Hg. Thus, the study of cold-induced hypertension in rats may allow a better understanding of the mechanisms involved in cold-induced elevation of blood pressure and hypertension in humans.

In conclusion, the present studies have demonstrated that chronic exposure to cold is a naturally occurring, reproducible model of hypertension, which does not appear to be unique to the laboratory rat. Further, although the exact mechanism by which cold induces hypertension was not discovered, the increased activity of the SNS, altered sensitivity of the baroreceptor reflex, and the transient increases in PRA, ACTH, B, E and altered volume status seen with chronic exposure to cold, suggest this to be a neurogenically-induced, renal-mediated form of hypertension. Many hypotheses have been formulated from these initial studies and remain for future investigation.

APPENDIX A
EXTRACTION OF PLASMA CATECHOLAMINES

Liquid Chromotagraphy Application Note No. 14
Bioanalytical Systems, Lafayette, IN

Reagents.

0.1 M $HClO_4$. Dilute 17.1 ml of 70% $HClO_4$ to 2 L.

3,4-dihydroxybenzylamine Internal Standard (DHBA). (100 ng/ml). Dissolve 16 mg of DHBA-HBr in 100 ml of 0.1 M $HClO_4$. Mix well, then dilute 100 ul of this stock solution to 100 ml with 0.1 M $HClO_4$. Keep refrigerated.

Norepinephrine (NE)/Epinephrine (E) Standard. (75 ng/ml NE, 25 ng/ml E). Dissolve 14.2 mg NE-bitartrate and 4.6 mg E-bitartrate in 100 ml of 0.1 M $HClO_4$. Mix well, then dilute 100 ul of this stock solution to 100 ml with 0.1 M $HClO_4$. Keep refrigerated.

Tris buffer/EDTA (1.5 M, pH 8.6). Combine 45 g of Tris base (Sigma, T-1503), 5 g Na_2EDTA and approximately 200 ml distilled water. Adjust pH with concentrated HCl dropwise until pH is 8.6. Stir until well dissolved. Dilute to 250 ml and keep refrigerated.

Phosphate buffer (0.1 M, pH 7.0). Dissolve 8.64 g Na_2HPO_4, 2.36 g KH_2PO_4 and 20.0 g Na_2EDTA in 1 L of distilled water.

Acid-washed aluminum oxide (AAO). Available from BAS in 10 g vials. (Lot No. 561).

Procedure.

1. Into a 5 ml conical vial, place the following: a) 0.5-2.0 ml plasma, b)phosphate buffer to adjust volume to 2 ml as needed, c) 25 ul DHBA, d) 50 mg AAO.

2. For each set of samples, prepare at least two standards as follows: a) 2.0 ml phosphate buffer, b) 25 ul DHBA, c) 50 ul NE/E standard, d) 50 mg AAO.

3. Add 1.0 Tris buffer, vortex, and shake immediately on a reciprocal shaker for 5 minutes. Add Tris buffer to each sample individually.

4. After shaking, allow the AAO to settle, then aspirate the supernatant.

5. Wash the AAO twice with distilled water, aspirating to near dryness each time.

6. Add 0.5-1.0 ml distilled water to each sample and transfer the AAO slurry with a disposable pipette to a microfilter loaded with RC-58 membrane (BAS, Lafayette, IN).

7. Place the microfilter into a centrifuge and spin the AAO to dryness at 1000 x g for 30 seconds.

8. Place a fresh reciever tube on the microfilter and add 200 ul of 0.1 M $HClO_4$ to each sample compartment. Vortex briefly. Let the sample stand for 5 minutes, vortex again.

9. Centrifuge the microfilter at 1000 x g for 1 minute. The acid extract in the receiver tube contains the catecholamines and is now ready for direct injection onto the column.

CALCULATIONS.

$$\text{(conc. NE)unk} = \frac{\text{(NE/DHBA)unk}}{\text{(NE/DHBA)known}} \times \text{(conc. NE)known}$$

PERFORMANCE PARAMETERS.

Linearity.

Figure A-1 describes the linearity of the system for NE, E, and DHBA over the 100-1500 pg range. Conditions were identical to those used in the assay of samples i.e. 2 nA full scale, 0.7 volts. A conservative estimate of the NE detection limit was approximately 100 pg/ml of extracted sample injected (Signal/Noise X 2 = 4).

Precision.

To test the precision of the assay, a pooled sample collected from donar rats was assayed in replicate as well as assayed in each sample run. Coefficients of variation were as followed: intraassay 6.5% and 10.4%, and interassay was 13 and 16% for NE and E, respectively. Calculations were made with DHBA as the internal standard.

APPENDIX B
ARGININE VASOPRESSIN RADIOIMMUNOASSAY

Arginine Vasopressin Extraction.

Procedure.

1. Prepare fresh bentonite solution (3 mg/ml ddH_2O) and place on magnetic stirer. Stir continuously.

2. Label 12 x 75 glass polypropylene tubes one for each sample.

3. Place 1 ml of sample into each tube, add 0.5 ml 1.0 N HCl and 1.0 ml bentonite solution. Keep ratio of sample/HCl/bentonite constant.

4. Shake or rock for 20 minutes.

5. Centrifuge at 3200 rpm for 30 minutes.

6. Aspirate supernatant, add 1 ml of acetone HCl solution (80:20).

7. Sonicate to breakup bentonite pellet and multivortex on maximum setting for at least 20 seconds.

8. Centrifuge 3200 rpm for 15 minutes.

9. Transfer supernatant to a new set of polypropylene tubes which contain 0.5 ml Petroleum ether.

10. Gently vortex and let stand 10 minutes.

11. Aspirate supernatant (Petroleum ether), then air dry overnight at room temperature.

12. Reconstituite in 600 ul buffer (300 ul buffer if initial sample was 500 ul).

Radioimmunoassay.

Reagents.

Assay buffer. (50 mM sodium phosphate, 10 mM EDTA, and 0.2% BSA). Add 1.787 g of Na_2HPO_4 (dibasic, FW 141.96), 1.341 g NaH_2PO_4 (monobasic, FW 155.99), 1.544 g EDTA (tetra sodium salt, FW 380.2) and 0.840 g of very good bovine serum albumin to 420 ml double distilled water.

^{125}I- Arginine Vasopressin. (Amersham, Arlington Heights, IL). Dilute in assay buffer to approximately 3000 cpm/25 ul.

Arginine Vasopressin Antibody. (Amersham, Arlington Heights, IL). Dissolve in 6.25 mls of assay buffer.

Charcoal Suspension. Add 1.0 g of washed activated charcoal (Sigma), 0.25 g Dextran (Kodak) to 100 ml of assay buffer. Mix for 30 minutes and store in refrigerator. Good for at least one month.

Procedure.

1. Label 12 x 75 polypropylene tubes in duplicate for total counts (TC), non-specific binding (NSB), blank (B_0), each of 8 standard, and each sample.

2. Make up fresh standards daily as follows: Dissolve 1 mg of AVP (Sigma) into 1 ml of assay buffer, (stock) divide into 100 ul aliquots and freeze until used.
   Use the stock standard of 100 pg/100 ul to make the following standard curve:
   0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 pg/tube.

3. Add 100 ul of reconstitued sample to each corespondingly labeled tube.

4. Add assay buffer as needed to NSB, B_0, and standards to bring total volume to 100 ul. Add an additional 25 ul to NSB.

5. Add 25 ul of anti-AVP to all tubes except TC and NSB.

6. Add 25 ul of ^{125}I-AVP to all tubes, vortex gently and incubate for 18-24 hours at 4° C.

7. Add 100 ul of cold, mixing charcoal suspension to each tube except TC. Immediately vortex, and centrifuge at 1,500 x g for 20 minutes.

8. Aspirate the supernatant which contains the antibody-bound ligand and count the pellet which contains the free-unbound ligand for 4 minutes in a gamma counter.

Results.

Plot B/B_0 against the concentration of AVP standards, then read pg unknown off the standard curve. Report results as pg/ml by 10. Cross-reactivity with related peptides was < 1%. Recovery of AVP from the extraction procedure was determined to be > 90%. The sensitivity of the assay is 0.15 pg/tube.

APPENDIX C
PLASMA RENIN ACTIVITY ASSAY

Angiotensin I Generation.

Reagents.

Inhibitor. In order, add: 0.225 g Phenylmethylsulfonyl Flouride (PMSF) (Sigma), 5.0 ml Ethanol (Fisher), and 0.025 ml 2,3-dimercaptopropanol (Sigma) to a 12 x 75 mm borosilicate tube. Cap and store at 4°C. Mix well before each use.

Generation Buffer. (0.5 M Citric acid, 1.0 M Na_2HPO_4, 5.0 mM EDTA and 3.08 mM NaN_3). Dissolve 5.252 g Citric acid (FW 210.1), 7.098 g Na_2HPO_4 (dibasic, FW 141.96), 0.073 g EDTA (disodium, FW 372.24) and 0.010 g NaN_3 (FW 65.01) into 50 ml of ddH_2O. Actual buffer pH should be 4.5, while the pH of the buffer/sample will be approximately 6.0. Store at 4°C. Buffer will keep for up to three months.

Procedure.

1. Turn on waterbath to 37°C.

2. Thaw the frozen samples on ice or in the refrigerator. Once thawed, the samples must be kept in an ice bath continually until they are ready to be refrozen.

3. For each sample label a set of polypropylene tubes, one in blue ink and one in black ink.

4. With each set of labeled tubes in ice baths add 0.100 ml of sample to the appropriate blue labeled tube.

5. Add 0.010 ml of generation buffer and 0.001 ml of inhibitor to each of the blue tubes. This is a 1:1.11 dilution of the plasma sample. Vortex for 10 seconds.

6. Transfer 0.050 ml from the blue tubes to the corresponding labeled black tubes.

7. Place the capped, black-labeled tubes in the water bath, incubate for 1.5 hrs. When the generation is complete, return the black labeled tubes to the ice bath. If the RIA is not done immediately on the generated and non-generated samples, they should be kept in an ice bath in the refrigerator until the RIA is run. * Do not freeze these samples *.

Radioimmunoassay of Angiotensin I

Reagents.

RIA Buffer. (0.05 M Trizma with 0.3 % BSA, pH=7.4). Dissolve 3.802 g Trizma 7.0 (Sigma), 1.47 g Bovine Serum Albumin (use 95% fraction or better, Sigma), and 0.049 g NaN_3 (FW 65.01) into 490 ml of double distilled water. Store at 4^o C. Buffer maybe kept for one month.

Angiotensin I Antibody. A final dilution of 1:60,000 was used. Mix for 30 minutes. Store at 4^oC.

^{125}I-Angiotensin I. (Amersham, Arlington Hts, IL). Dilute in RIA buffer to approximately 6000 cpm/0.05 ml. Mix for 30 minutes. Store at 4^oC.

Charcoal Suspension. Add 1.0 g of washed activated charcoal (Sigma), 0.25 g Dextran (Kodak) to 100 ml RIA buffer. Mix for 30 minutes. Store at 4°C. Good for at least one month.

Angiotensin I Standard. Dissolve angiotensin I (Sigma) in RIA buffer to 100 ng/ml. Store frozen in 0.200 ml aliquots.

Procedure.

1. Keep all sample and assay tubes in ice bath throughout RIA setup.

2. Label polypropylene tubes in duplicate for total counts (TC), non-specific binding (NSB), blank (B_0), each of 8 standards, and each sample. Each sample actually consists of a generated sample (black tube) and a non-generated sample (blue tube) so that there will be 4 tubes labeled for each of the original samples.

3. Make working standards using the following scheme:

   Add 0.200 ml of 100 ng/ml A I to 1.800 ml RIA buffer= 10 ng/ml= A
   Add 1.000 ml A to 1.000 ml buffer= 5 ng/ml= B
   Add 1.000 ml B to 1.000 ml buffer= 2.5 ng/ml= C
   Add 1.000 ml C to 1.000 ml buffer= 1.25 ng/ml= D
   Add 1.000 ml D to 1.000 ml buffer= 0.625 ng/ml= E
   Add 1.000 ml E to 1.000 ml buffer= 0.312 ng/ml= F
   Add 1.000 ml F to 1.000 ml buffer= 0.156 ng/ml= G
   Add 1.000 ml G to 1.000 ml buffer= 0.078 ng/ml= H

4. Make the additions to the assay tubes according to the scheme below.

tube #	RIAbuffer	sample	standard	antibody	125I-A I
1,2(TC)					0.050 ml
3,4(NSB)	0.110 ml				0.050 ml
5,6(B_o)	0.060 ml			0.050 ml	0.050 ml
7-22(std)	0.010 ml		0.050 ml	0.050 ml	0.050 ml
23-26(pl)	0.050 ml	0.010 ml		0.050 ml	0.050 ml
27-	0.050 ml	0.010 ml		0.050 ml	0.050 ml

** For stimulated samples (high PRA values), dilution of the generated sample may be required.

5. Vortex and incubate overnight at 4°C.

6. With the tubes and spinning charcoal mixture (start spinning 15 minutes prior to use) in an ice bath, add 0.250 ml of the charcoal to all the tubes except the TC tubes. Keep the addition of charcoal to all the tubes under 1 minute. Immediately vortex all the tubes. Let stand for 3 minutes then centrifuge for 25 minutes.

7. Aspirate the supernatant from all tubes except the TC tubes.

8. Count for 4 minutes on gamma counter.

Precision.

Two distinct pools were run with each assay and were used to calculate interassay coefficient of variability of 9.1%.

Limit of detection.

The limit of detection of the assay was defined as 90% of Bo and was found to be 0.1235 ng/ml of AI.

Reference

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APPENDIX D
CORTICOSTERONE RADIOIMMUNOASSAY

Reagents.

Assay Buffer. Heat, but do not boil, 1 liter of distilled water. Add 2.0 g gelatin to heated water. After the gelatin has dissolved, add 18.0 g NaCl (FW 58), 17.4 g Na_2HPO_4 (FW 142), 2 g Na azide (FW 65), and 10.8 g NaH_2PO_4 (FW 138) and mix until its in solution. Cool to room temperaure and adjust total volume of 2 liters with distilled water. Unadjusted pH should be 7.0 ± 0.1. Store covered in refrigerator.

Charcoal Suspension. Dissolve 62.5 mg Dextran-T70 (Kodak) in 100 ml of assay buffer. Add 625 mg Norit A (Sigma) and mix vigorously. Cover and store in refrigerator for up to 1 month.

Counting solution. Ecoscint A, (National Diagnostics).

Standards. Dissolve 10 mg of standard in 100 ml absolute ethanol for a concentrated solution of 100 ug/ml. Dilute 1:100 (0.5 ml in 50 ml) in ethanol for 1 ug/ml stock standard in volumetric flask. Store both stock solutions well stoppered and sealed with Parafilm in the freezer.

Working standards: made up fresh daily.

A	100 ul	stock	+ 9.90 ml assay buffer	= 5.00 ng/0.5 ml
B	2.0 ml	5.0 ng/0.5 ml	+ 3.00 ml assay buffer	= 2.00 "
C	1.0 ml	5.0 ng/0.5 ml	+ 4.00 ml assay buffer	= 1.00 "
D	0.5 ml	5.0 ng/0.5 ml	+ 4.50 ml assay buffer	= 0.50 "
E	0.2 ml	5.0 ng/0.5 ml	+ 4.80 ml assay buffer	= 0.20 "
F	0.1 ml	5.0 ng/0.5 ml	+ 4.90 ml assay buffer	= 0.10 "
G	0.05 ml	5.0 ng/0.5 ml	+ 4.95 ml assay buffer	= 0.05 "
H	0.02 ml	5.0 ng/0.5 ml	+ 4.98 ml assay buffer	= 0.02 "
I	0.01 ml	5.0 ng/0.5 ml	+ 4.99 ml assay buffer	= 0.01 "

^{3}H-Corticosterone. (New England Nuclear). Take 5 ul of trace and evaporate to dryness under nitrogen. Add 20 ml of assay buffer and allow to stand at room temperature for 30 minutes prior to use. Dilution equals approximately 15,000 cpm/ 0.1 ml. Store in refrigerator.

Corticosterone Antibody. (Radioassay Systems Laboratories, Inc.) Catalog # 147, shipped as raw sera. Dilute 0.0667 ml raw sera into 100 ml assay buffer for a working dilution of 1:1500. Final assay dilution is 1:10,500.

Procedure.

1. Label 10 x 75 mm disposable glass culture tubes in duplicate for total counts (TC), non-specific binding (NSB), blank (B_0), each of 8 standards, and each sample.

2. Make the additions to the assay tubes according to the scheme below. Add samples, standard and assay buffer to each tube and vortex gently. Cover and incubate all tubes in a water bath at 80° C for 20 minutes. Allow to cool completly, approximately 5 minutes, then proceed with addition as follows:

tube	#buffer	sample	standard	antibody	trace
1,2 (TC)	610 ul				100 ul
3,4 (NSB)	610 ul				100 ul
5,6 (B_0)	510 ul			100 ul	100 ul
7-22 (std)	10 ul		500 ul	100 ul	100 ul
23-26 (pool)	500 ul	10 ul		100 ul	100 ul
27-	500 ul	10 ul		100 ul	100 ul

vortex gently, cover and incubate overnight in refrigerator.

3. Place container of charcoal suspension in an ice bath on a magnetic stirrer. Stir vigorously.

4. Place all tubes in an ice bath.

5. Add 200 ul of charcoal suspension to each assay tube except total counts.

6. Vortex and let sit on ice for 20 minutes.

7. Centrifuge at 1500 x g for 20 minutes.

8. Decant the entire supernatant into mini-vials and add 5 mls of scintillant.

9. Count for 5 minutes on beta counter.

Results.

Plot B/B_0 against the corticosterone standards, then read ng unknown off the standard curve. Report results as ug/100 ml (ug%) by multipling 10 ul sample by 10.

Precision.

Interassay coefficients of variation were determined from 2 different pools run in each assay and were as follows: pool 1 = 12.2%, pool 2 = 7.9%.

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BIOGRAPHICAL SKETCH

Paula Elizabeth Papanek was born January 26, 1958, in Hinsdale, Illinois. She is the second of four children. Her older sister, Mary Ann Papanek-Miller obtained her M.F.A. from The University of Houston. Mary Ann is a professional artist and teacher living in Houston. Her younger sister, Carolyn, graduated from the University of Wisconsin, Stout, with a degree in fashion merchandise. Carolyn is in Management at Bloomingdales Inc., Chicago. Her younger brother John, will graduate from the University of Louisville in August of 1989 with a degree in psychology. Paula graduated from Hinsdale Central High School in 1975. In September of 1975, she entered the College of DuPage, Glen Ellyn, Illinois, for one quarter. In January of 1976, she was selected to the United States Womens' National Volleyball Team, where she competed and trained for 20 months. In September of 1977, she accepted an athletic scholarship at Illinois State University, Normal, Illinois. She received a B.S. degree in nutritional biochemistry, as part of the contract major degree program in May of 1983.

From August of 1981 to May of 1983, she was a Graduate Assistant and Assistant Womens' Volleyball Coach in the Department of Athletics at the University of Louisville. In January of 1982, she was admitted to degree status in the Department of Exercise Physiology in the College of Allied Health. In July of 1983, she was awarded a Graduate Research Assistantship in Exercise Physiology. In May of 1984, she received a M.S. degree in exercise physiology from the University of Louisville. She proceeded to the University of Florida and entered the graduate program in physiology in August of 1984.

Under the supervision of Dr. Melvin J. Fregly, Paula Elizabeth Papanek was awarded a Ph.D. in May, 1989. She intends to pursue a challenging career in research investigating the interaction between the central nervous system and the kidney in the homeostatic regulation of the cardiovascular system.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Melvin J. Fregly

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Graduate Research Professor of
Physiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1989

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